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I am submitting herewith a thesis written by Elizabeth Diana Barker entitled "The Synthesis and Characterization of a Novel Polysaccharide Hydrogel for Biomedical Applications Including the Treatment of Malignant Tumors and the Prevention of Metastatic Disease." I have examined the final paper copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Polymer Engineering.

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The Synthesis and Characterization of a Novel Polysaccharide Hydrogel for Biomedical Applications Including the Treatment of Malignant Tumors and the Prevention of Metastatic Disease

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee at Knoxville

Elizabeth Diana Barker
August 2007

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Dedication

I would like to dedicate this work to all of you who have helped me become the person I have always wanted to be, a contributor. Thank You.

He who travels alone travels faster.

The eyes of the master fattens the cow.

Sleep is practicing for death.

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Abstract

Amygel® is an injectable biodegradable controlled release drug delivery system with adjustable release kinetics determined by varying the concentrations of the polymeric components of the material. Amygel® is a starch based biodegradable hydrogel consisting of an interpenetrating network of physically entangled amylose and amylopectin polymer chains crosslinked with d-glucaric acid. Amylose and amylopectin are the two components of naturally occurring starch. The immiscibility of the amylose and amylopectin due to differences in solubility parameter and chain size induces phase separation within the network but because the chains entangled during hydrolysis an interpenetrating network is formed. The chemically reactive carboxyl groups of the d-glucaric acid react with the –OH groups of the amylose and amylopectin chains via condensation creating an ester linkage. This ester linkage degrades according to the same hydrolytic mechanism of the main chain backbone resulting in the release of di-acid while the hydrolysis of the acetal bonds of the amylose and the amylopectin results in the generation of glucose monomers, maltose dimers, and maltotriose trimers which can all be safely consumed by the surrounding cells in the tissue. Raman Spectroscopy confirms the formation of ester linkages with the addition of d-glucaric acid to starch gels. With the addition of the chemical crosslinker, the elastic modulus of the starch hydrogel increases. Also, with increased crosslink concentration, the degradation time of the system is extended. D-glucaric acid is a proven anti-carcinogenic agent, and there is evidence that Amygel® inhibits the cell proliferation of osteosarcomas by up to 70%.

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I. Introduction

Recent advancements in medicine have gone beyond oral and injectable systemic delivery of therapeutic pharmaceutical agents to treat disease and are currently pursuing avenues of drug delivery directly to the site of affected organs, tissues and cells. Polymer Engineering and Biomaterials research has contributed greatly to the areas of medicine and pharmaceutical science through the development of novel materials and material systems that can be used as vehicles of drug delivery. The system described in this paper is a novel biodegradable hydrogel made of natural polymers that has two-fold drug delivery capabilities. One, the material has the ability to complex with pharmaceutical compounds, so the gel can be loaded with a specific drug to be delivered to treat a particular disease at a targeted site . Two, the degradation product of the chemical crosslinker of the hydrogel is a proven therapeutic agent effective against carcinogenic cells.

Carcinogenesis

The induction of cancer depends on inherited and acquired susceptibility factors, on exposure to initiation factors, and on promotion and progression factors. Malignant neoplasia is the result of multiple genetic defects successively accumulating over a period of time. It is now apparent that the cellular evolution to malignancy involves the sequential alteration of proto-oncogenes and/or tumor suppressor genes whose gene products participate in critical pathways for the transduction of signals and/or regulation of gene expression [Walaszek 2004]. These stages have been defined experimentally as initiation, promotion, and progression.

Initiation involves mutation of cellular DNA resulting in the activation of oncogenes and the inactivation of tumor suppressor genes [Walaszek 2004]. Initiation is thought to be irreversible and consists of a single gene mutation that is caused in most cases by environmental genotoxic agents such as chemicals, radiation, and viruses. Oncogenes can also be activated by chromosomal translocations and gene amplifications [Walaszek 2004].

Promotion follows initiation and involves the process of gene activation, such that the latent phenotype of the initiated cell becomes expressed through cellular selection and clonal expansion. This can occur through a variety of mechanisms including toxicity, terminal differentiation or mitotic inhibition of the noninitiated cells, and mitogenesis of the initiated cells [Walaszek 2004]. While promotion occurs over a long period of time, it is reversible in its early stages. Proof that promotion is reversible in humans is supported by the observation that the rate of lung cancer induction in individuals who quit smoking approaches that of nonsmokers [Walaszek 2004]. The breadth of the available data, as well as the multistage nature of tumor promotion suggests that this process may involve the interaction of a number of endogenous factors as well as environmental factors such as chemicals, radiation, viruses, bacteria, and diet and nutrition. In human cancer, smoking, alcoholic beverages, diet and nutrition and environmental factors such as asbestos, hydrocarbons, radiation, and hormones are now thought to have more of a promotional influence in the multistage process of carcinogenesis [Walaszek 2004].

The last step leading to cancer is called progression. Progression involves genetic damage that results in the conversion of benign cells into malignant neoplasms capable of invading adjacent tissues and metastasizing to distant sites. The additional genetic

alterations thought to be required for neoplastic progression often occur faster than would be expected from the statistics of accidental genotoxic insults due to so-called genetic instability. The concept of genetic instability implies that while environmental genotoxic agents generally cause cancer initiation, the additional mutations required for neoplastic progression may be attributed to endogenous reactions and factors such as detoxification and removal of damaged cells by programmed cell death [Walaszek 2004]. Genetic instability may be a result of errors in DNA replication or spontaneous hydrolytic alterations of DNA such as depurination and deamination in combination with an impaired ability of pre-malignant cells to repair DNA damage. Modified DNA bases, especially 8-hydroxy-2'-deoxyguanosice, produced by oxygen-free radicals have been implicated in the initiation of cancer. The importance of free radicals in radiation carcinogenesis and oxygen-free radicals and electrophiles in chemical carcinogenesis is also well recognized [Walaszek 2004].

Chemoprevention, namely inhibition or reversal of carcinogenesis, may be conducted at a variety of time points in this process to reduce occurrence of invasive cancers - primary intervention at earlier stages in the process or secondary intervention at later stages in the process. Chemoprevention applies to the prevention of clinical cancer by the administration of pharmaceuticals or dietary constituents. The efficacy of such preventive interventions is evaluated in clinical trials. Phase I trials determine the dose-related safety of drugs and frequently include pharmacokinetic studies. Phase II and III trials are used to test drug activity. Agents used in successful Phase II clinical trials have evidence of chemopreventive efficacy and the high likelihood of the agent preventing

cancer at the target site [Walaszek 2004]. All experimental treatments must have a high margin of safety and a logical presumed mechanism of chemopreventive activity.

The mechanisms that focus on initiation events include (a) inhibition or alteration of phase I enzymes responsible for the formation of reactive carcinogenic metabolites; (b) inhibition or induction of oxidative enzyme pathways that produce products of lower carcinogenic potential; (c) induction of detoxification enzymes, phase II enzymes and pathways for both proximate and ultimate carcinogen; (d) scavenging of reactive carcinogenic intermediates through direct chemical interaction; (e) inhibition or enhancement of DNA repair mechanisms; and (f) inhibition of cell proliferation and DNA synthesis [Hanausek 2003]. Realistically, there is a higher degree of success when treated with combinations of compounds that can act through one or more different mechanisms.

Table 1 lists a number of potent inhibitors of tumor initiation that appear to be effective because they prevent the formation of the ultimate carcinogen [Walaszek 2004]. Some phenolic antioxidants have been extensively studied, primarily because of their use as food preservatives. In addition to the alterations in oxidative metabolism, the phenolic antioxidants have also been shown to increase the detoxification pathways for many chemical carcinogens. The indoles, aromatic isothiocyanates, coumarines, flavones, di and triterpenoids, dithiothiones, organosulfides, chlorophyllin, and D-glucarates have a potent effect on the body's metabolism of carcinogens [Walaszek 2004]. In general, they appear to have a major effect on the detoxification of carcinogens. Ellagic acid, 2,6-dithiopurin, and chlorophyllin have been also shown to be highly potent in scavenging the ultimate reactant carcinogenic form of carcinogens [Walaszek 2004]. Disulfiram, vitamin

Table 1. Table of Anti-tumor Compounds According to Mechanism of Inhibition.

Prevention Strategy	Examples
Tumor Initiation	
-Inhibit carcinogen activity	Epigallocatechin gallate (EGCG), Selenium, Phehtyl-isothiocyanate (PEITC), Indol-3-carbinol, Coumarins, Ellagic Acid, Resveretrol, Genistein, 1-ethynylpyrene
-Scavenge electrophiles	Ellagic Acid, EGCG, 2,6-dithiopurine, Chlorophyllin
-Enhance Carcinogen Detoxfication	Oltipraz, Diallyl Sulfide (DAS), EGCG, PEITC, N-acetylcysteine (NAS), D-glucarate, Resveratrol
-Enhance DNA repair	Calorie Restriction, EGCG, Selenium
Tumor Promotion / Progression	
-Scavenge Reactive Oxygen Species	Antioxidants (Carotenoids, α-tocopherol, Ascorbic Acid, Chlorophyllin, α-lipoic Acid, Proanthocyanidins, EGCG, Avicins, Selenium, Calorie Restriction)
-Alter Gene Expression	Retinoids (*all-trans* Retinoic Acids, Fenretinide), Calorie Restriction, Monoterpenes, Fluasterone, Dehydroepiandrosterone (DHEA)
-Decrease Inflammation	Steroidal anti-inflammatory, Non-steroidal Anti-inflammatory drugs (NSAIDs), Calorie Restriction, DHEA, Fluasterone, Antihistamines
-Suppress Proliferation	Calorie Restriction, Selenium, DHEA, Difluoromethylomithine (DFMO), Tamoxifen, Genistein, Fluasterone, Retinoids, D-glucarate, Steroidal Anti-inflammatory agents
Induce Differentiation	Retinoids, Calcium, Sodium Butyrate
-Encourage Apoptosis	DHEA, Fluasterone, Fenretinide, Sodium Butyrate, Curcumins, 2-methoxy-extradiol
-Detoxify Steroid Hormones	D-glucarate, Aromatase Inhibitors, Anti-estrogens such as Tamoxifen

C, and vitamin E appear to inhibit chemical carcinogenesis in a similar manner to the phenolic antioxidants by their effect on the metabolism of the carcinogen, their antioxidizing activity, and preventing the formation of carcinogens. The mechanisms by which selenium inhibits chemically induced tumors may be related to its effect on the glutathione peroxidase since it is a cofactor for this enzyme [Walaszek 2004].

The process of tumor promotion and progression involves a combination of several mechanisms. Among anti-tumor promoting mechanisms, Table 1, the ones that are most promising include the following: inhibition of inflammation, inhibition of cell proliferation and hyperplasia; modulation of cell differentiation and apoptosis; scavenging of reactive oxygen species and preventing depletion of anti-oxidant defense systems; and enhancement of tumor promoter detoxification pathways [Walaszek 2004]. The natural and synthetic cancer preventive agents that exert their effects against tumor promotion usually inhibit one, more, or even all events involved in the tumor promotion process [Walaszek 2004]. It is unclear however, whether inhibition of one particular event involved in promotion by chemopreventive agents is sufficient to exert their maximum to complete anti-tumor promoting effects.

Natural compounds found in plant products may prevent carcinogenesis by interfering with the actions of mutagens, carcinogens, and tumor promoters. These natural compounds are effective inhibitors of tumor initiation, promotion, and or progression. Their mechanism of action is related to their abilities to prevent critical carcinogen metabolism and to increase detoxification of carcinogens and tumor promoters. The potential role of the detoxification system and the roles of d-glucaric acid have been studied [Hanausek 2003].

The potential role of a detoxification system and the roles of D-glucaric acid and β-glucuronidase in early detection and prevention of cancer have been investigated [Hanausek 2003]. β-Glucuronidase is one of many pro-carcinogenic enzymes. D-glucaric acid is a natural, apparently nontoxic compound produced by some plants, especially in fruits and vegetables, and in small amounts by mammals, including humans. D-glucaric acid an end product of d-glucuronic acid or its lactone leads to oxidation products that hydrolyze spontaneously in aqueous solution to give the potent beta-glucuronidase inhibitor, d-glucaro-1,4 lactone, noninhibitory d-glucaro 6,3 lactone, and d-glucaric acid, all of which are excreted in urine. D-glucaric acid was identified as a normal constituent of urine and serum. In fact, d-glucaric acid is a major serum organic acid. However, significant differences in serum levels and urinary excretion of d-glucaric acid have been reported in healthy patients. Since urinary excretion of d-glucaric acid increases following exposure to xenobiotics, it was suggested for use as an indirect indicator of hepatic microsomal enzyme induction by xenobiotic agents. D-glucaric acid urinary excretion in cancer patients and tumor-bearing rats was found to be significantly lower than in healthy controls. In mice with experimental tumors and in cancer patients, uninvolved liver had a lowered d-glucaric acid level [Hanausek 2003]. Cancer tissue itself lacked the d-glucaric acid synthesizing system.

The physiological function of D-glucaric acid is unclear. Formation of D-glucaro-1,4 lactone, and inhibitor of beta-glucuronidase , from one of the products of its hydrolytic action, could be regarded as a negative feedback mechanism. The accumulation of free carcinogens, tumor promoters, and other toxins normally excreted as glucuronides may be aggravated not only by the elevated beta-glucoronidase activity

but also by the depressed synthesis of glucaric acid. D-glucaro-1,4 lactone does not directly affect the uridine 5'-diphospho-glucuronosyltransferase activity. However, by inhibiting beta-glucoronidase, it does enhance net glucuronidation. Therefore, it has a potential for chemoprevention of cancer [Hanausek 2003]. There is now extensive evidence from short-term and long-term models for the possible control of different stages of carcinogenic process by the beta-glucoronidase inhibitor D-glucaro-1,4-lactone, and specifically by its precursors such as d-Glucaric acid salts (D-glucarates) [Walaszek 2004]. A phase I clinical trial was undertaken to begin to explore the potential role of D-glucaric acid in the prevention of lung cancer in tobacco smokers. K-ras oncogene mutations were present in some of the smokers in the lung cancer study, but there was no evidence of K-ras oncogene mutations in non-smokers implying that K-ras mutations may be involved in the development of malignant tissues. D-glucaric acid is thought to interfere in the K-ras pathway thereby preventing the conversion of benign cells to malignant ones.

Oral doses of glucaric acid were shown to inhibit beta-glucoronidase. In current human studies, d-glucaric acid is administered in the form of calcium-d-glucarate. The glucarate is ingested orally and the acidic environment of the digestive system converts the salt to the acid form. The d-glucaric acid is then metabolized into 3 isomeric forms- d-glucaric acid, d-glucaro-1,4 lactone, and d-glucaro 6,3 lactone. These compounds are absorbed through the lining of the gastrointestinal tract, enter the blood stream, are circulated throughout the body, and then excreted in the urine [Thorne 2002]. The implications of these findings are that K-ras mutations may be involved in the development of malignant tissues, and d-glucaric acid is thought to interfere with the K-ras pathway thereby preventing the conversion of benign cells to malignant ones.

Drug Delivery Systems

A therapeutic agent interacts with the body at one of three sites: within the circulation or interstitial space, at membrane receptors on the surface of the cell, or at various sites of cellular machinery within the cell. A wide variety of polymeric biomaterials are routinely used for delivering drugs to these sites [Ratner 2004]. The major advantage of developing systems that release drugs in a controlled manner is illustrated in Figure 1 which shows blood plasma levels following a single dose administration of a therapeutic agent. When drugs are taken orally, the blood plasma concentration rapidly rises and then exponentially decays as the drug is metabolized and eliminated from the body [Ratner 2004]. The figure also shows drug concentrations above which the drug could be toxic or have adverse effects and below which it is not therapeutically effective. The difference between these two levels is known as the therapeutic index.

Increasing the size of the dose does increase the time during which the concentration of the drug is above the minimum effective concentration, however this can also increase the chances of approaching toxic blood plasma concentrations and side effects. Desired drug concentration can also be maintained by periodic doses, but this is inconvenient and usually is impeded by poor patient compliance. For these reasons, there have been great interest in developing controlled-release formulations and devices that can maintain a desired blood plasma level for long periods of time without reaching a toxic level or dropping below the minimum effective level. These systems are called "zero order" systems because they release the drug at a constant rate [Ratner 2004].

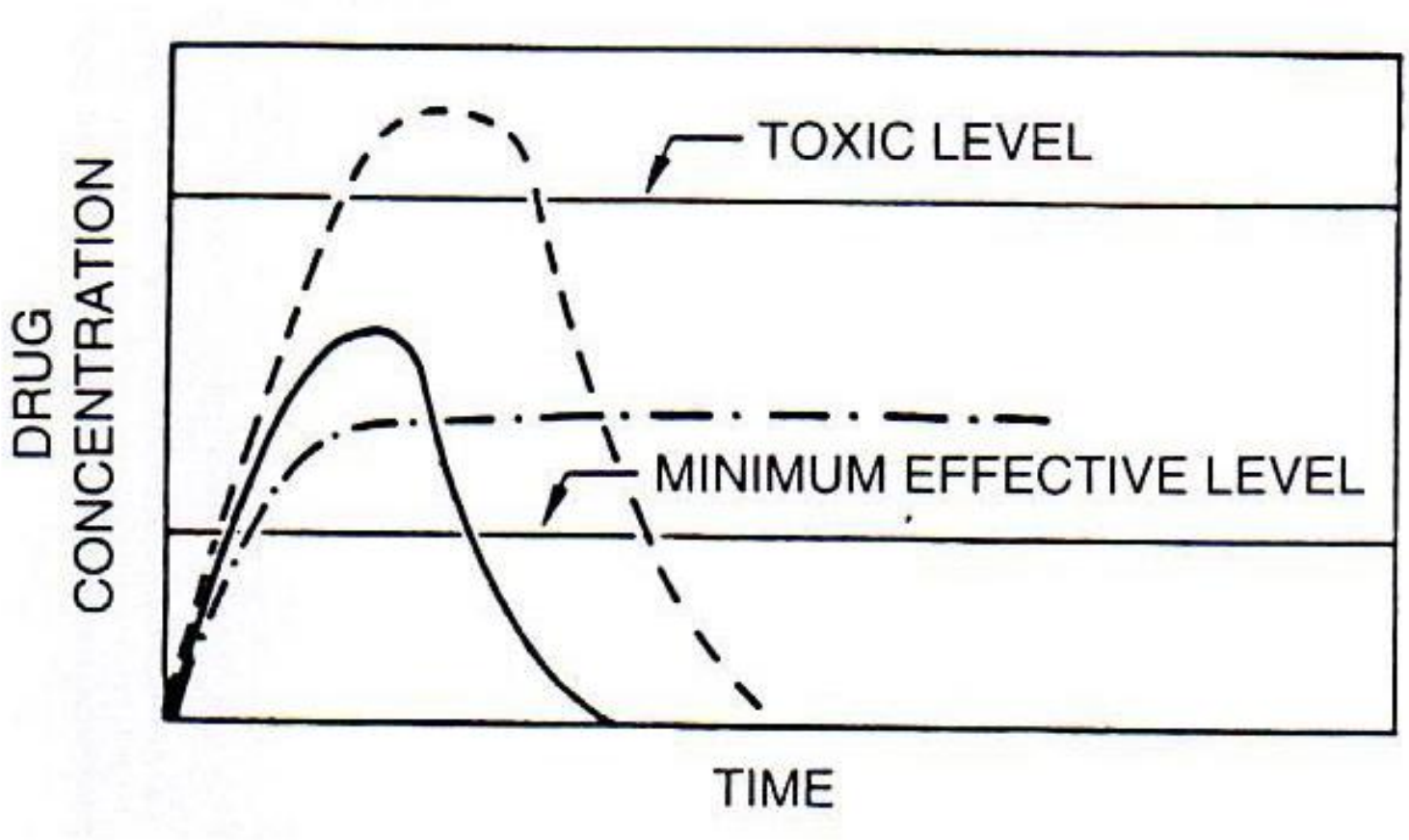


Figure 1. Changes in Drug Concentration as a Function of Time.

Research to successfully formulate pharmaceuticals to extend the activity of therapeutic agents began as early as the 1940's. The first product to minimize the frequency between oral doses was Spansules ®. This consisted of small drug spheres encapsulated with a soluble coating. By varying the thickness of the coating, the capsule dissolution times were extended thus prolonging the action of one dose of the drug. Today, these types of formulations are now known as "sustained release" or "prolonged release" and most recently "controlled-delivery" capsules [Ratner 2004].

The functionality of these types of sustained release systems greatly depends on the local *in vivo* environment which varies from patient to patient. To reduce patient-to-patient variability, a major effort to develop products capable of releasing drugs by predictable and reproducible delivery kinetics was begun in the late 1960's. Such devices are known as "controlled-release" devices. There are four types of controlled release systems- Drug Diffusion, Water Penetration, Swelling, and Chemical [Ratner 2004].

Drug Diffusion Controlled Release Systems

There are two fundamentally different devices where the rate of drug release is controlled by drug diffusion. These are membrane-controlled devices and monolithic devices. In a membrane controlled system, the active agent is contained in a core or reservoir which is surrounded by a thin polymer membrane. The release into the surrounding environment occurs by diffusion through the membrane. The rate of diffusion through the membrane is a function of the polymer membrane usually the porosity, the thickness, or the chemical reactivity [Ratner 2004].

When the membrane is not porous, diffusion can be described by Fick's first law:

$$J = -D\frac{dCm}{dx} \qquad (1)$$

Where J is the flux per unit area, in g/cm^{2}s, Cm is the concentration of the agent in the membrane in g/cm^3, dCm/dx is the concentration gradient, and D is the diffusion coefficient of the agent in the membrane in cm^2/s [Ratner 2004].

Because the concentration of the agent in the membrane cannot be readily determined, Equation 1 can be rewritten using partition coefficients which describe the ratio of the concentration of the agent in the membrane when it is at equilibrium with the concentration in the surrounding medium.

$$J = \frac{DK\Delta C}{l} \qquad (2)$$

where ΔC is the difference in the concentration between the solutions on either side of the membrane, K is the partition coefficient, and l is the thickness of the membrane [Ratner 2004].

Reservoir devices, such as the adhesive patch illustrated below, can also be constructed with membranes that have well-defined pores that permeate through the membrane.

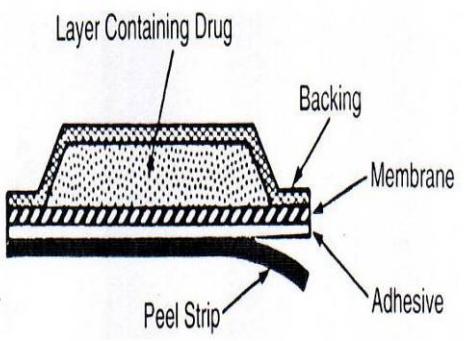


Figure 2. Schematic of Reservoir Delivery Device.

Diffusion in such micro-porous membranes occurs principally by diffusion through the liquid-filled pores where the composition of the liquid will control the overall transport flux across the membrane. In this system, the flux is described by Equation 3

$$J = \frac{\varepsilon DK\Delta C}{\tau \cdot l} \qquad (3)$$

where ε is the volume fraction porosity, which is normalized as fractional area of pores per unit area of the membrane, and τ is the tortuosity the average length of pore channel through the membrane [Ratner 2004].

One of the most important consequences of Equations 2 and 3 is that the flux J will remain constant provided that all parameters remain constant including that the membrane material does not change with time. Another requirement for zero-order delivery is also that ΔC remain constant. Also, the concentration of the agent in the core cannot change with time and the agent released from the device has to be rapidly removed, this is known as the sink condition. Constant agent concentration in the core can be attained by dispersing the agent in a medium in which it has a very low solubility leading to a saturated drug concentration in the reservoir [Ratner 2004]. However, preventing

drug build up around the device is not always possible. Agents having very low water solubility often deviate from zero-order kinetics because of "boundary layer effects."

Another factor that impedes constant drug release is migration of the agent from the core into the membrane during storage. When this happens and the device is then placed in the release medium, the initial drug release is more rapid than expected because the agent diffuses from the saturated membrane. The release rate then slows down until the steady-state, zero-order, release rate is achieved [Ratner 2004]. This initial nonlinear release is known as the "burst effect."

In a monolithic device the therapeutic agent is uniformly dispersed or dissolved in a polymer matrix and the release is controlled by diffusion of the drug from the matrix. The matrix is assumed to be inert, either non-swelling or fully swollen, and non-degrading. Mathematical treatment of this type of diffusion depends on the solubility of the agent in the polymer. This model is dependent on one of two assumptions. One, the agent is present below its solubility limit and is dissolved in the polymer. Two, the agent is present well above its solubility limit and is dispersed in the polymer [Ratner 2004].

For an agent that is dissolved in the polymer, release can be calculated by two equations, known as early time approximation and late time approximation

$$\frac{dM_t}{dt} = 2M_x\left[\frac{D}{\pi \cdot l^2 \cdot t}\right]^{\frac{1}{2}} \qquad (4)$$

$$\frac{dM_t}{dt} = \frac{8DM_x}{l^2}\exp\frac{\pi^2 Dt}{l^2} \qquad (5)$$

These equations predict rate of release from a slab of thickness l, where D is the diffusion coefficient, M_x is the total amount of agent dissolved in the polymer, and Mt is

the amount released at time t. According to Equation 4, which is valid over about the first 60% of the release time, the rate decreases linearly with the square root of time. During the latter 40% of the release time the rate decays exponentially with time, as shown by Equation 5. Plots of these two approximations are shown in Figure 3 [Ratner 2004] When the agent is dispersed as particles in the polymer, release kinetics can be calculated by the Higuchi equation for a planar slab model system.

$$\frac{dM_t}{dt} = \frac{A}{2}\left[\frac{2DC_sC_o}{t}\right]^{\frac{1}{2}} \qquad (6)$$

where A is the slab area, C_s is the solubility of the agent in the matrix, and C_o is the total concentration of dissolved and dispersed agent in the matrix. In this particular case, release rate decreases as the square root of time over the major portion of the delivery time and deviates only after the concentration of the active agent remaining in the matrix falls below the saturation value C_s [Ratner 2004].

Water Penetration Controlled Release Systems

Water Penetration Controlled Delivery Systems are systems where the drug release rate is controlled by the rate of water diffusion into the device. The two types of water penetration controlled systems are Osmotic and Swelling Controlled Systems.

Osmotically controlled devices operate according to the Figure 4.
In this device, an osmotic agent is contained within a rigid housing and is separated from the drug by a movable partition. One wall of the rigid housing is a semipermeable membrane, and when the device is placed in aqueous environment water is osmotically drawn across the semipermeable membrane. The resultant increase in volume within the

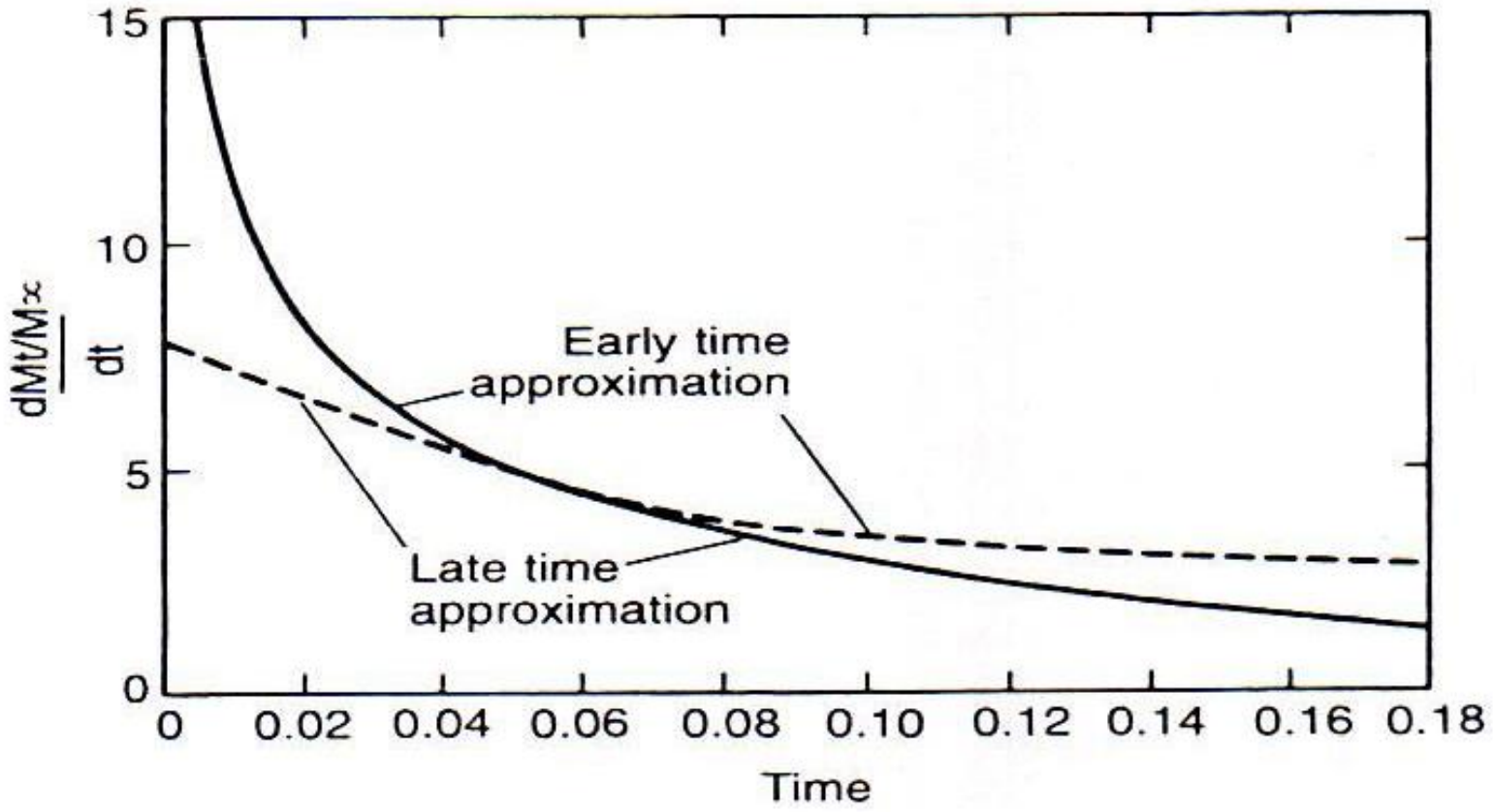


Figure 3. Release Kinetics of a Monolithic Delivery Device.

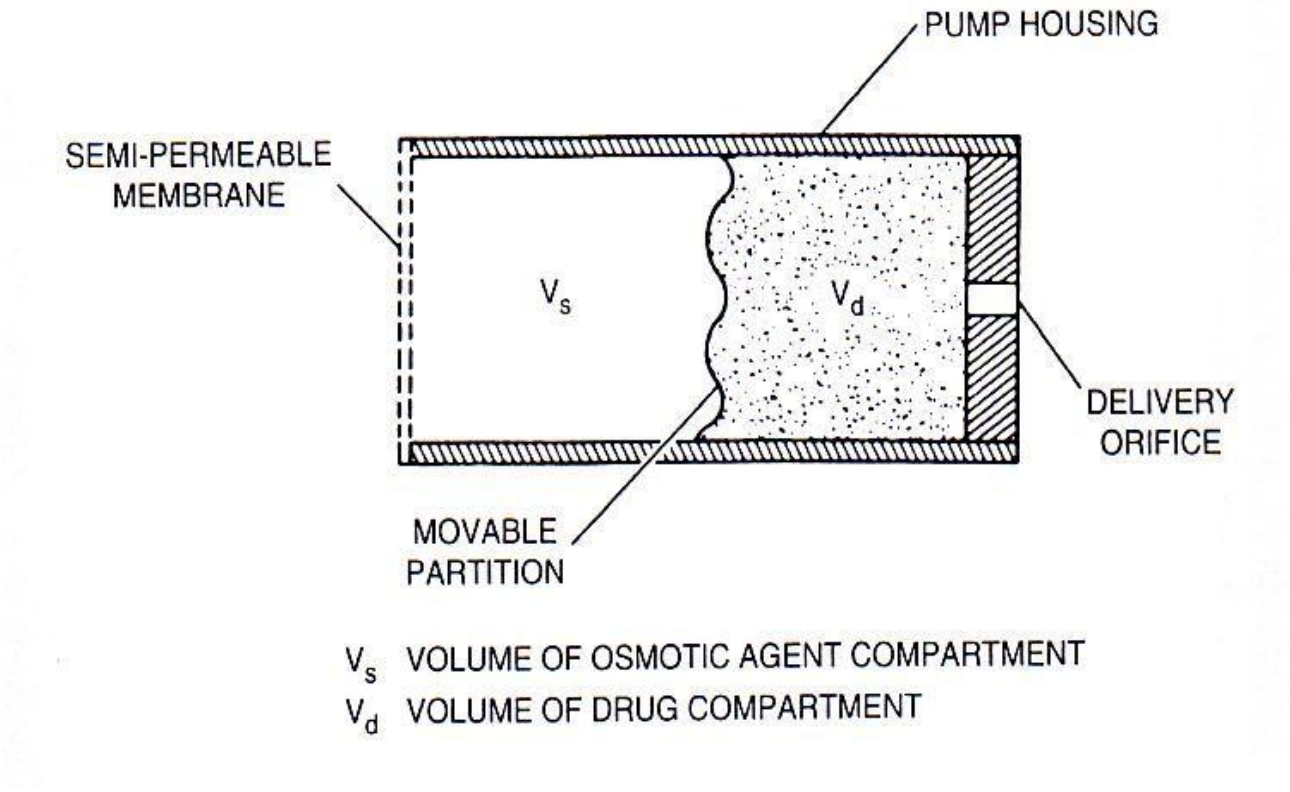


Figure 4. Schematic of Osmotically Controlled Delivery Device.

osmotic compartment exerts pressure on the partition which forces the agent out of the device through the delivery orifice [Ratner 2004].

The volume flux of water across the semipermeable membrane is given by:

$$\frac{dV}{dt} = \frac{A}{l} L_p \left[\sigma \Delta\pi - \Delta P\right] \qquad (7)$$

where $\frac{dV}{dt}$ is the volume flux, $\Delta\pi$ and ΔP are, respectively the osmotic and hydrostaticpressure differences across the semipermeable membrane, L_p is the membrane mechanical permeability coefficient, σ is the reflection coefficient, approximately 1, and A and *l* are the membrane area and thickness respectively

The mass rate of delivery $\frac{dM}{dt}$ of the agent is then given simply by

$$\frac{dM}{dt} = \frac{dV}{dt} C \qquad (8)$$

where C is the concentration of the agent in the solution that is pumped out of the orifice [Ratner 2004].

Swelling Controlled Release Systems

In swelling controlled devices, the agent is dispersed in a hydrophilic polymer that is stiff or glassy in the dehydrated state and when placed in aqueous environment will swell and absorb the solvent from the environment. Because diffusion of molecules in a glassy matrix is extremely slow, no significant release occurs while the polymer is in the glassy state. However, when the material is placed in an aqueous environment, water will penetrate the matrix and as a consequence of swelling the drug diffuses out of the matrix and is released from the polymer [Ratner 2004].

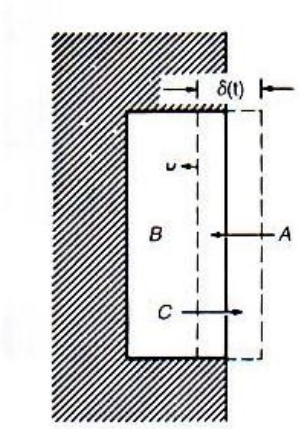


Figure 5. Schematic of a Swelling Controlled Delivery Device.

The process shown schematically above (figure 5) is characterized by the movement of two fronts [Ratner 2004].

B is a block of glassy polymer in the dehydrated state. One front, the swelling interface (A), separates the glassy polymer from the swollen rubbery polymer and moves inward into the device at a velocity, v. The other front, the polymer interface (C), moves outward as it swells, and separates the swollen polymer from the pure dissolution medium. The polymer may go through a gel state as it swells. In systems where the glassy polymer is non-crystalline and linear, dissolution takes place. When the polymer is highly crystalline or crosslinked, no dissolution takes place and the swollen polymer remains as a hydrogel [Ratner 2004].

Chemically Controlled Release Systems

Chemically controlled delivery systems are divided into two categories: degradable reservoir and drug dispersed in a biodegradable matrix. The degradable reservoir system contains the drug in the core of a solid implant covered by a

biodegradable membrane. This delivery system is identical to the reservoir system except that the membrane surrounding the drug core is bioerodible or biodegradable. Such systems combine the advantage of long-term zero-order drug release with bioerodibility or biodegradability. Because constancy of drug release requires that the diffusion coefficient D of the agent in the membrane remain constant, the bioerodible membrane must remain essentially unchanged over the duration of drug delivery. Typically, the bioerodible polymer and the drug are both hydrophobic. The membrane must remain intact while there is still drug in the core to prevent its abrupt release. Thus, significant bioerosion cannot take place until drug delivery has been completed. The second type of chemically controlled system is the most common because the drug is dispersed in a biodegradable matrix and release of the drug is determined by the rate of degradation of the polymer holding the drug in the matrix [Ratner 2004].

Biodegradation

The development of an implantable biodegradable drug delivery system is probably the most widely investigated application of degradable polymers because the device will eventually run out of drug or the need for the delivery will be eliminated once the disease has been treated [Ratner 2004]. One of the most important prerequisites for the successful use of a degradable polymer for any medical application is a thorough understanding of the way the device will degrade or erode and resorb from the implant site into the body. The transformation of such an implant into water soluble materials is best described by the term bioerosion. The bioerosion process of a solid polymeric implant is associated with macroscopic changes in the device's mechanical properties such as swelling, deformation or structural disintegration, weight loss, and the eventual

depletion of the drug and loss of function. Each of these properties represents distinct and often independent aspects of the complex bioerosion behavior of a specific polymer. It is important to note that the bioerosion of a solid device is not necessarily due to the chemical cleavage of the polymer backbone or the chemical cleavage of crosslinks or side chains [Ratner 2004].

Two distinct modes of bioerosion have been described in the literature, bulk erosion and surface erosion. In bulk erosion, the rate of water penetration into the solid device exceeds the rate at which the polymer is transformed into water-soluble monomers. Consequently, the uptake of water is followed by an erosion process that occurs throughout the entire volume of the solid device. Because of the rapid penetration of water into the matrix of hydrophilic polymers, most of the currently available polymers will give rise to bulk eroding devices. In a typical bulk erosion process, cracks and crevices will form throughout the device that may result in the device rapidly crumbling into pieces. A good illustration for a typical bulk erosion process is the disintegration of an aspirin tablet that has been placed in water. Depending on the specific application, the often uncontrollable tendency of bulk eroding devices to crumble is a disadvantage [Ratner 2004].

In surface erosion, the bioerosion process is concentrated at the surface of the device. Therefore, the device will become thinner with time but maintain its structural integrity throughout much of the erosion process. In order to observe surface erosion, the polymer must be hydrophobic to prevent the rapid penetration of water into the interior of the device. Because the drug will be released as the polymer swells, the rate at which the polymer degrades into water soluble monomers is slower than the rate of water

penetration into the device, so step one is to swell and release the drug then after the polymer has served its function to release the drug it then degrades. Scanning electron microscopic evaluation of surface eroding devices has sometimes shown a sharp border between the eroding surface layer and the intact polymer in the core of the device.

Surface eroding devices have so far been obtained only from a small number of hydrophobic polymers containing hydrolytically degradable linkages in the backbone. A possible exception to this general rule is enzymatic surface erosion. The inability of enzymes to penetrate into the interior of a solid polymeric device may result in a concentrated enzyme-mediated surface erosion mechanism [Ratner 2004].

Although bioerosion can be caused by the solubilization of an intact polymer, chemical degradation of the polymer is usually the underlying cause for bioerosion of a solid polymeric device. There are several distinct types of chemical degradation mechanisms that have been identified. Chemical reactions can lead to cleavage of crosslinks between water-soluble polymer chains, to the cleavage of polymer side chains resulting in the formation of polar or charged groups, or to the cleavage of the polymer main chain backbone. Combinations of these reactions are also possible. For example, a cross-linked polymer may first be partially solubilized by the cleavage of crosslinks, followed by the cleavage of the backbone itself. Water is the key to all of these degradation schemes. Even enzymatic degradation occurs in aqueous environment [Ratner 2004].

A hydrogel is a crosslinked polymeric system essentially forming one continuous network and this network has the ability to absorb solvent without damage to the network. Hydrogels in pharmaceutical applications have become popular in recent years

because of their biocompatibility [Ratner 2004]. Pharmaceutical hydrogel systems include matrices that have a drug incorporated into them and are swollen to equilibrium. There are two types of the solvent-activated matrix release devices: swellable and swelling-controlled. A system prepared by incorporating a drug into a hydrophilic polymer can be swollen when brought in contact with water or biological fluids, but this swelling process may or may not be the controlling mechanism for diffusional release. Release is determined by the relative rates of the macromolecular relaxation of the polymer and drug diffusion from the gel [Ratner 2004].

In swelling controlled release systems, the bioactive agent is dispersed into the polymer to form nonporous films, disks, or spheres and upon contact with an aqueous dissolution medium a distinct front or interface occurs where the water solvent front penetrates into the polymer and separates the solid polymer from the rubbery, gel-like, state of the material. Under these conditions, the macromolecular relaxations of the polymer influence the diffusion mechanism of the drug through the rubbery state. The swelling process proceeds toward equilibrium at a rate dependent on the water activity in the system and the structure of the polymer. If the polymer is crosslinked or if it is of sufficiently high molecular weight so that chain entanglements can maintain structural integrity, the equilibrium state is a water swollen gel. The equilibrium water content of such hydrogels can very from 30% to 90%. If the dry hydrogel contains a water soluble drug, the drug is essentially immobile in the matrix, but begins to diffuse out as the polymer swells in the water solvent. Drug release then depends on two simultaneous rate processes: water migration into the device and drug diffusion outward through the swollen gel [Ratner 2004].

Hydrogels are crosslinked polymeric structures formed by either covalent bonds produced by the simple reaction of one or more comonomers, physical crosslinks from entanglements, association bonds such as hydrogen bonds or strong van der Waals interactions between chains, or crystallites bringing together two or more macromolecular chains that are able to swell in water. Hydrogels can be categorized by their method of preparation, ionic charge, or structural features. Hydrogels can be homopolymer, copolymer, multipolymer, or interpenetrating polymeric networks depending on the method of preparation. Homopolymer hydrogels are crosslinked networks of one type of hydrophilic monomer unit. Copolymer hydrogels are produced by crosslinking two comonomer units. Multipolymer hydrogels are produced from three or more comonomers that have been reacted together to form a network. Interpenetrating networks are usually formed by producing the first network and then swelling that network in the other monomer. The monomer then reacts to form a second network structure that is intertwined with the first network. Because of monomer functionalities and chemical nature of the functional groups within the chains, charge effects can occur producing neutral, anionic, cationic, or ampholytic gels. Crosslinked networks can have amorphous, semi-crystalline or complexed physical structures. In amorphous hydrogels, the macromolecular chains are arranged randomly. Semicrystalline hydrogels have dense regions of ordered macromolecular chains, or crystallites, that are arranged randomly. Complexed structures are those formed by hydrogen bonds or chemical complexes of one monomer with another or of the chains of the network [Ratner 2004].

The terms crosslink and junction or tie-point indicate the connection points of the polymer chains that make up the network. Hydrogel forming crosslinks may be carbon

atoms but they are usually small chemical bridges with molecular weights much smaller than those of the polymer chains. A crosslink may also be an association of macromolecular chains caused by van der Waals forces or an aggregate formed by hydrogen bonds [Ratner 2004]. Aggregates are masses of physically entangled or weakly bound polymer chains that serve as junction points in the formation of a 3 dimensional network.

Methods of preparation of the initial networks include chemical crosslinking, photopolymerization, or irradiative crosslinking. Hydrogels are produced by swelling crosslinked structures in water or biological fluids. Because during preparation hydrogels must be brought in contact with water to yield the final swollen network structure, their physical behavior is largely dependent on their equilibrium and dynamic swelling behavior. Usually, a hydrophilic crosslinked network is placed in water, and because of the thermodynamic compatibility of the macromolecular chains interacting with the solvent molecules the network then expands to its swollen state [Ratner 2004].

The Flory-Huggins theory can be used to determine thermodynamic values related to the mixing process. Flory's model describing the equilibrium of crosslinked polymer networks proposed that the degree to which a polymer network is able to swell is determined by the elastic retractive forces of the polymer chains and the thermodynamic compatibility of the polymer with the solvent molecules. In terms of the free energy of the system, the total free energy change of the system upon swelling is described as

$$\Delta G = \Delta G_{elastic} + \Delta G_{mix} \qquad (9)$$

In this equation, $\Delta G_{elastic}$ is the contribution due to the elastic retractive forces and ΔG_{mix} represents the thermodynamic compatibility of the polymer and the swelling agent, water [Ratner 2004].

Upon differentiation of Equation 9 with respect to the water molecules in the system, an expression can be derived for the chemical potential change of water in terms of the elastic and mixing contributions due to swelling [Peppas, Biomaterials]

$$\mu_1 - \mu_{1,0} = \Delta\mu_{elastic} + \Delta\mu_{mix} \qquad (10)$$

Here, μ_1 is the chemical potential of water within the gel and $\mu_{1,0}$ is the chemical potential of pure water [Ratner 2004].

At equilibrium, the chemical potentials of water inside and outside of the gel must be equal. Therefore, the elastic and mixing contributions to the chemical potential will balance one another at equilibrium. The chemical potential change upon mixing can be determined from the heat of mixing, and the entropy of mixing. Using the Flory-Huggins theory, the chemical potential of mixing can be expressed as:

$$\Delta\mu_{mix} = RT\left(\ln\left(1 - 2v_{2,s}\right) + v_{2,s} + \chi_1 v_{2,s}^2\right) \qquad (11)$$

Where χ_1 is the polymer water interaction parameter, $v_{2,s}$ is the polymer volume fraction of the gel, T is absolute temperature, and R is the gas constant [Flory].

This thermodynamic swelling contribution is counterbalanced by the retractive elastic contribution of the crosslinked structure. The elastic contribution of the crosslinked structure is described by the rubber elasticity theory. Equilibrium is defined when these two forces are equal in a given solvent and at a specific temperature. The volume degree of swelling, Q, is the ratio of the actual volume of a sample in the swollen

state divided by its volume in the dry state, and can be calculated using Equation 12 [Ratner 2004].

$$v_{2,s} = \frac{Volume\ of\ \ polymer}{Volume\ of\ \ swollen\ gel} = \frac{V_p}{V_{gel}} = \frac{1}{Q} \qquad (12)$$

Determination of the swelling characteristics of hydrogels is important to their biomedical and pharmaceutical applications because the equilibrium degree of swelling influences the surface properties and surface mobility, the optical properties, the mechanical properties, and the solute diffusion coefficient through these hydrogels [Ratner 2004].

The parameter that describes the basic structure of the hydrogel is the molecular weight between crosslinks, Mc. This parameter defines the average molecular size between two consecutive junctions regardless of the nature of those junctions and can be calculated by Equation 13 [Ratner 2004]

$$\frac{1}{\overline{M}_c} = \frac{2}{\overline{M}_c} - \frac{\left(\frac{v}{V_1}\right)\left[\ln\left(1 - v_{2,s}\right) + v_{2,s} + \chi_1 v_{2,s}^2\right]}{\left(v_{2,s}^{\frac{1}{3}} - \frac{v_{2,s}}{2}\right)} \qquad (13)$$

The second parameter of importance in structural analysis of hydrogels is the crosslinking density, ρ_x, which is the number of crosslinks in the system and is described by Equation 14

$$\rho_x = \frac{1}{v\overline{M}_c} \qquad (14)$$

In these equations, v is the specific volume of the polymer, the reciprocal of the amorphous density of the polymer, and M_n is the initial molecular weight of the uncrosslinked polymer [Ratner 2004].

The mechanical behavior of hydrogels is best understood using the theories of rubber elasticity and viscoelasticity. These theories are based on time independent and time dependent recovery of the chain orientation structure. By using theories to describe the mechanical behavior, it is possible to analyze the polymer structure and determine the effective molecular weight between crosslinks as well to gain insight about the number of elastically active chains and cyclization versus crosslinking tendencies. In many instances it is not possible to test the hydrogel under the exact conditions in which the device is used, and for this reason it is of particular importance to use theories to extrapolate properties at these conditions [Anseth 1996].

The dependence of mechanical properties on the chemical properties of the polymer corresponds to typical methods used to examine polymers in a material selection process: the monomers used, the polymerization conditions, the crosslink density, the degree of swelling, and the type of medium in which the material is swollen. There are advantages and disadvantages of varying each of these parameters with respect to the ultimate hydrogel mechanical properties.

Rubbers are materials that respond to stresses with nearly instantaneous and fully reversible deformation. Whereas glasses can be reversibly stretched only up to ~1%, rubbers typically exhibit reversible behavior up to ~1000% elongation. Normal rubbers are lightly crosslinked networks with a rather large free volume that allows them to respond to external stresses with a rapid rearrangement of the polymer segments. In their swollen state, most hydrogels satisfy these criteria for a rubber. When a hydrogel is in the region of rubber-like behavior, the mechanical behavior of the gel is dependent mainly on the architecture of the polymer network. General characteristics of rubber

elastic behavior include high extensibility generated by low mechanical stress, complete recovery after removal of the deformation, and high extensibility and recovery that are driven by entropic rather than enthalpic changes [Anseth 1996].

To derive relationships between the network characteristics and the mechanical stress-strain behavior, classical thermodynamics, statistical thermodynamics, and phenomenological approaches have been used to develop an equation of state for rubber elasticity [Anseth]. From classical thermodynamics, the equation of state for rubber elasticity may be expressed as

$$f = \left(\frac{\partial U}{\partial L}\right)_{T,V} + T\left(\frac{\partial f}{\partial T}\right)_{L,V} \qquad (15)$$

Where f is the retractive force of the elastomer in response to a tensile force, U is the internal energy, L is the length, V is the volume, and T is the temperature. For ideal rubber elastic behavior, the first term in Equation 15 is zero; bonds are not stretched with changes in L. This behavior is not true for most other materials, such as metals, where changes in length cause internal energy driven retractive forces. For elastomeric materials, an increase in length brings about a decrease in entropy because of changes in the end-to-end distances of the network chains. The retractive force and entropy are related through the following Maxwell equation

$$\left(\frac{\partial S}{\partial L}\right)_{T,V} = \left(\frac{\partial f}{\partial T}\right)_{L,V} \qquad (16)$$

Figure 6 graphically depicts these quantities for the thermodynamic equation of state for rubber elasticity.

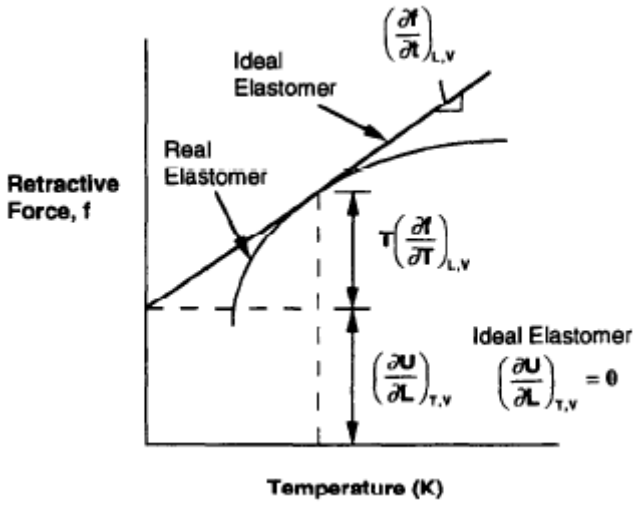


Figure 6. Retractive Force as a Function of Temperature of an Elastic Network.

Stress-strain analysis of the energetic and entropic contributions to the retractive force (Equation 15) indicates that entropy accounts for more than 90% of the stress. Thus, the entropic model for rubbery elasticity is a reasonable approximation.

From statistical thermodynamics, the retractive force of an ideal elastomer may be expressed as

$$f = -T\left(\frac{\partial S}{\partial L}\right)_{T,V} = -kT\left(\frac{\partial \ln \Omega(r,T)}{\partial r}\right)_{T,V} \qquad (17)$$

Here, k is the Boltzmann constant, r is a certain end-to-end distance, and Ω (r,T) is the probability that the polymer chain with and end-to-end distance r at temperature T will adopt a certain conformation. Equation 17 assumes that the internal energy contribution to the retractive force is constant or zero. Only the entropy contributions to the retractive force are considered. Evaluation of Equation 17 for a single chain leads to

$$f = \frac{3kTr}{\overline{r_f^2}} \qquad (18)$$

where r_f^2 is the end-to-end distance in the bulk state for linear chains.

If the analysis is expanded to n network chains, then

$$\Delta A_{el} = \frac{3nkT}{\overline{r_f^2}} \int_{\left(\overline{r_1^2}\right)^{1/2}}^{\left(\overline{r^2}\right)^{1/2}} r\,dr \qquad (19)$$

Here ΔA_{el} is the change in Helmholtz free energy caused by elastic deformation. After the integration and assuming no volume change upon deformation, the statistical thermodynamic equation of state for rubber elasticity is obtained below [Anseth 1996].

$$\tau = \left(\frac{\partial A}{\partial \lambda}\right)_{T,V} = \frac{\rho RT}{M_C} \frac{\overline{r_o^2}}{\overline{r_f^2}} \left(\lambda - \frac{1}{\lambda^2}\right) \qquad (20)$$

Here, τ, is the shear stress per unit area, ρ is the density of the polymer, Mc is the number average molecular weight between crosslinks, and λ is the extension ratio. The quantity, r_o/r_f, is the front factor and is the ratio of the end-to-end distance in a real network versus the end-to-end distance of isolated chains. In the absence of knowledge concerning these values, the front factor is often approximated as 1.

From Equation 20, the elastic stress of a rubber under uniaxial extension is directly proportional to the number of network chains per unit volume (ρ/Mc). This equation assumes that the network is ideal in that all chains are elastically active and contribute to the elastic stress. Network imperfections such as cyclic structures, chain entanglements, and chain ends are not taken into account. To correct for chain ends

$$\tau = \frac{\rho RT}{M_c} \frac{\overline{r_o^2}}{\overline{r_f^2}} \left(1 - \frac{2\overline{M_c}}{\overline{M_n}}\right) \left(\lambda - \frac{1}{\lambda^2}\right) \qquad (21)$$

where M_n is the number average molecular weight of the linear polymer chains before crosslinking. This correction becomes negligible when $M_n >> M_c$.

From constitutive relationships, the shear modulus G is then

$$G = \frac{\rho RT}{\overline{M_c}} \frac{\overline{r_0^2}}{\overline{r_f^2}} \left(1 - \frac{2\overline{M_c}}{\overline{M_n}}\right) \tag{22}$$

and the force per unit area is

$$\tau - G\left(\lambda - \frac{1}{\lambda^2}\right). \tag{23}$$

Note the dependence of the shear modulus on M_c. Also, the stress-strain behavior of rubbery elastic materials is nonlinear. Figure 7 illustrates the typical experimental behavior, along with the statistical thermodynamic predictions.

While the thermodynamic and statistical thermodynamic approaches describe observed rubber-elastic behavior at low extensions quite well, the equations are less applicable and invalid at higher elongations (λ>3).

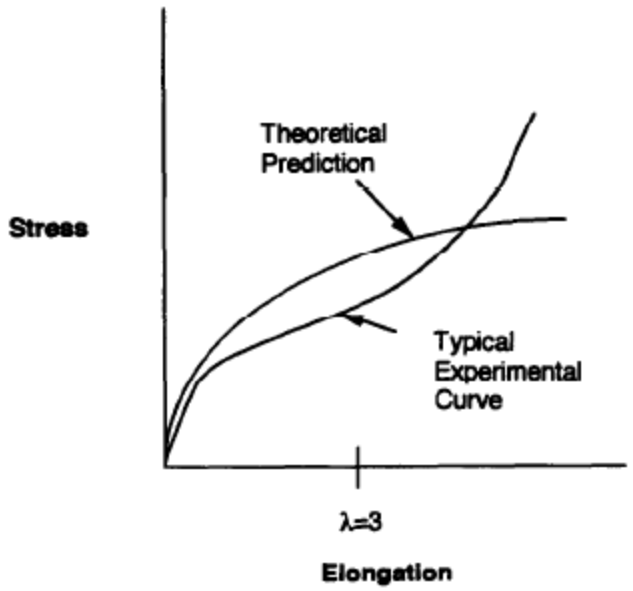


Figure 7. Stress vs. Elongation Curves of an Elastic Network.

Equation 21 can be modified to fit the mechanical behavior of a swollen rubber

$$\tau_s = N_0 v_r^{1/3} RT \left(\frac{\overline{r_0^2}}{\overline{r_f^2}}\right)\left(\lambda_s - \frac{1}{\lambda_s^2}\right) \tag{24}$$

where τ_s and λ_s are the shear stress and elongation measured in the swollen state. Here, v_r is the ratio of the unswollen volume to the swollen volume or the polymer volume fraction, and N_o is the effective number of crosslinks determined from

$$\frac{\rho}{\overline{M}_c}\left(1 - \frac{2\overline{M}_c}{\overline{M}_n}\right) = N_0 v_r^{1/3}. \tag{25}$$

Therefore, for a given hydrogel at a fixed temperature, a higher degree of swelling results in a reduction of the stress. Alternatively, if the degree of crosslinking is increased the stress is increased.

At very high extension ratios, the thermodynamic equations of state are less satisfactory and phenomenological treatments are used. The phenomenological approach does not consider the molecular structure of the gel and is concerned only with the observed macroscopic behavior of the system. The fundamental issue is to develop an equation for the energy stored in the polymer. The most general form of an equation for the work that is stored in the body of an isotropic material as strain energy, W, is a power series expression [Anseth 1996]

$$W = \sum_{i,j,k=0}^{\infty} C_{ijk}(I_1 - 3)^i (I_2 - 3)^j (I_3 - 1)^k. \tag{26}$$

Here, I1,I2, and I3 are the strain invariants given by

$$I_1 = \alpha_1^2 + \alpha_2^2 + \alpha_3^2 \tag{27}$$

$$I_2 - \alpha_1^2\alpha_2^2 + \alpha_2^2\alpha_3^2 + \alpha_3^2\alpha_1^2 \tag{28}$$

$$I_3 = \alpha_1^1\alpha_2^2\alpha_3^2 \tag{29}$$

The Mooney-Rivlin equation, which is an expression describing uniaxial extension of an incompressible material, retains two of the terms in the series given in Equation 26. The first is the lowest member of the series, i=1,j=0, and k=0, and the second term is i=0,j=1, and k=0. For the case of uniaxial extension and letting C_1=C_{100} and C_2=C_{010}, one obtains the Mooney-Rivlin equation [Anseth 1996]

$$\tau = \left(\frac{\partial W}{\partial \lambda}\right) = 2\left(C_1 + \frac{C_2}{\lambda}\right)\left(\lambda - \frac{1}{\lambda^2}\right) \tag{30}$$

The equation of state from statistical thermodynamic analysis given in Equation 21 predicts that the quantity τ/λ-$1/\lambda^2$ should be constant, whereas the Mooney-Rivlin equation is able to better predict the stress-strain behavior at higher elongations.

Equation 30 can be modified to describe the behavior of a swollen hydrogel, and this expression is given below [Anseth 1996]

$$\tau_s = 2C_1 v_r^{1/3}\left(\lambda_s - \frac{1}{\lambda_s^2}\right) + 2C_2 v_r^{5/3}\left(1 - \frac{1}{\lambda_s^3}\right) \tag{31}$$

The viscoelasticity theory considers the relationships between elasticity, flow, and molecular motion in polymeric materials. While the mechanical behavior of all materials exhibit some degree of elasticity and flow, the size of polymer molecules often lead to a viscoelastic response. The magnitude of the viscoelastic response is strongly dependent

on the nature of the imposed mechanical motion. For example, the viscoelastic response is at a maximum when the time scale of the mechanical motion is on the time scale of the mechanical motion of the polymer. In general, hydrogels are not simply elastic materials, but behave viscoelastically. The time dependence of the applied stress or strain is as important as the magnitude in predicting the material's mechanical response.

An applied mechanical stress or strain leads to a time dependence on the strain or stress as the segments of the polymer chains move. This movement instigates an internal response that results in a time dependent recovery when the initial condition is removed. If the recovery is complete at long times, the behavior is termed viscoelastic. If the recovery is incomplete, the behavior is called viscoelastic-viscoplastic. In general, a polymer may have several viscoelastic processes each associated with a particular molecular motion in the material.

If a constant shear stress, is applied to a viscoelastic material the shear strain is observed to be time dependent as shown in Figure 8. The ratio of the time dependent shear strain to the applied stress is defined as the creep compliance, J(t). If J(t) is measured over long times, J(t) shows little time dependence at very short and very long times. At very short times, the time scale of the mechanically induced stress is much shorter than the time scale for the molecular motions in the polymer. The system remains unrelaxed and the creep compliance is J_u, and when the material is completely relaxed the creep compliance is J_r. Because it is easier to vary the experimental temperature than the frequency, it is common to present data as a series of isochronals, with each curve representing data obtained at a specific frequency. If a number of isochronals are obtained, the linear viscoelastic region can be identified. In the linear region, J(t) is

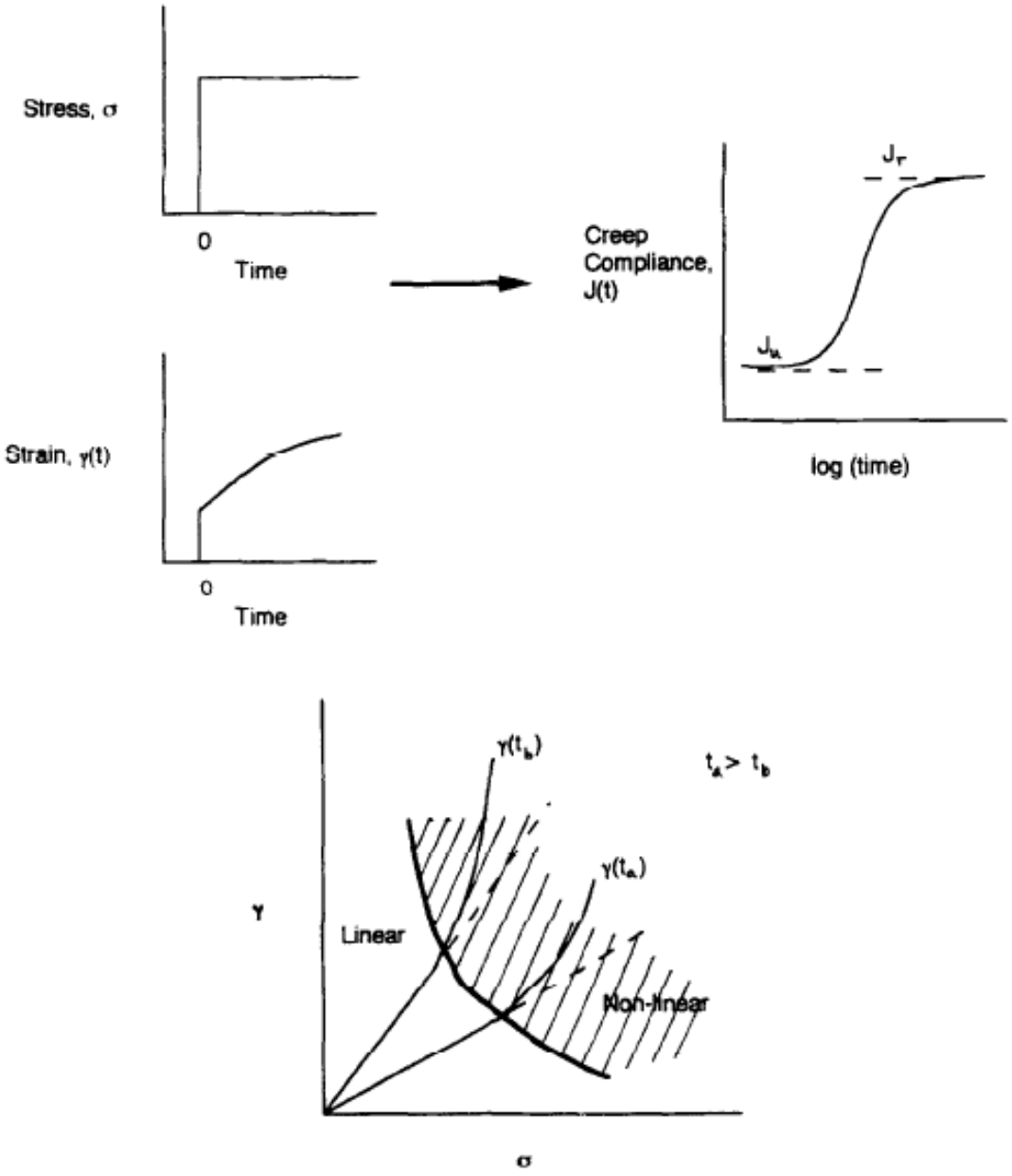


Figure 8. Stress Strain Curves of an Elastic Material Under Constant Stress.

independent of the magnitude of the stress that is imposed. For nonlinear-viscoelastic behavior, each value of the applied stress leads to a time-dependent strain which can only be determined by an experiment conducted at that stress.

A constant shear strain is applied and the time dependence of the stress required to maintain that strain is measured. The experiment is illustrated in Figure 9. The stress relaxation modulus, G(t), is defined as the stress-strain ratio at constant deformation. As in the case of creep, several isochronals can be plotted to determine the range of linear stress relaxation behavior. Unlike creep, nonlinear behavior leads to slower stress relaxation, In certain regions, J(t) and G(t) have a nearly reciprocal relationship, especially at short times and for crosslinked systems at long times as well.

Rheological analysis provides quantitative information on the viscoelastic properties of a material by measuring the mechanical response of a sample as it is deformed. Generalized input and response curves are depicted in Figure 10.
A common notation for sinusoidal tests is the complex dynamic modulus, G*, defined below

$$G^* = G' + iG'' = \frac{\sigma^*}{\gamma^*}, \tag{32}$$

Here, G' is the real, also elastic or storage modulus and G'' is the imaginary also viscous or loss modulus. These definitions apply to testing in the shear mode where G refers to the shear modulus, σ' to the shear stress, and γ' to the shear strain.

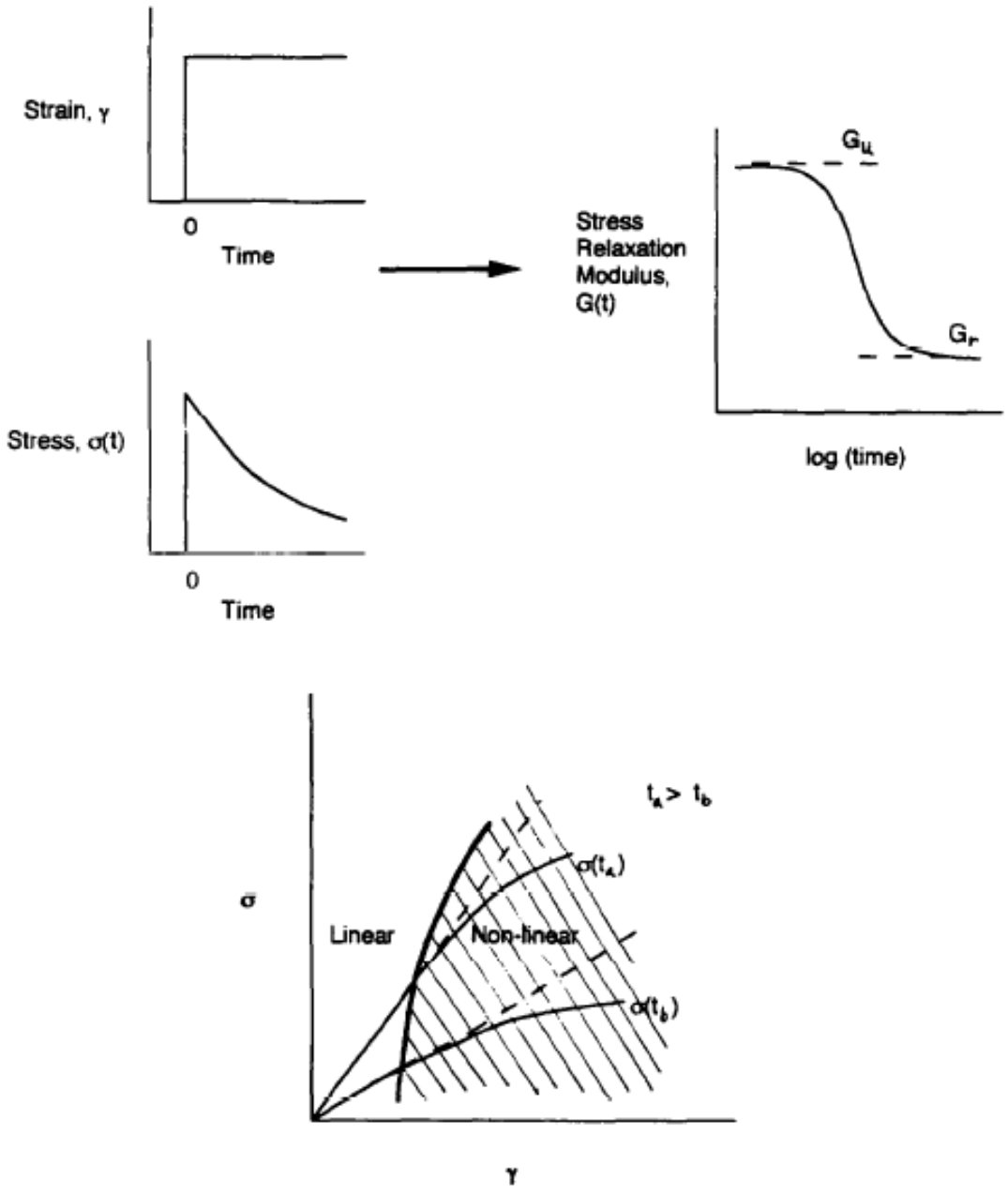


Figure 9. Stress Strain Curves of an Elastic Material Under Constant Strain.

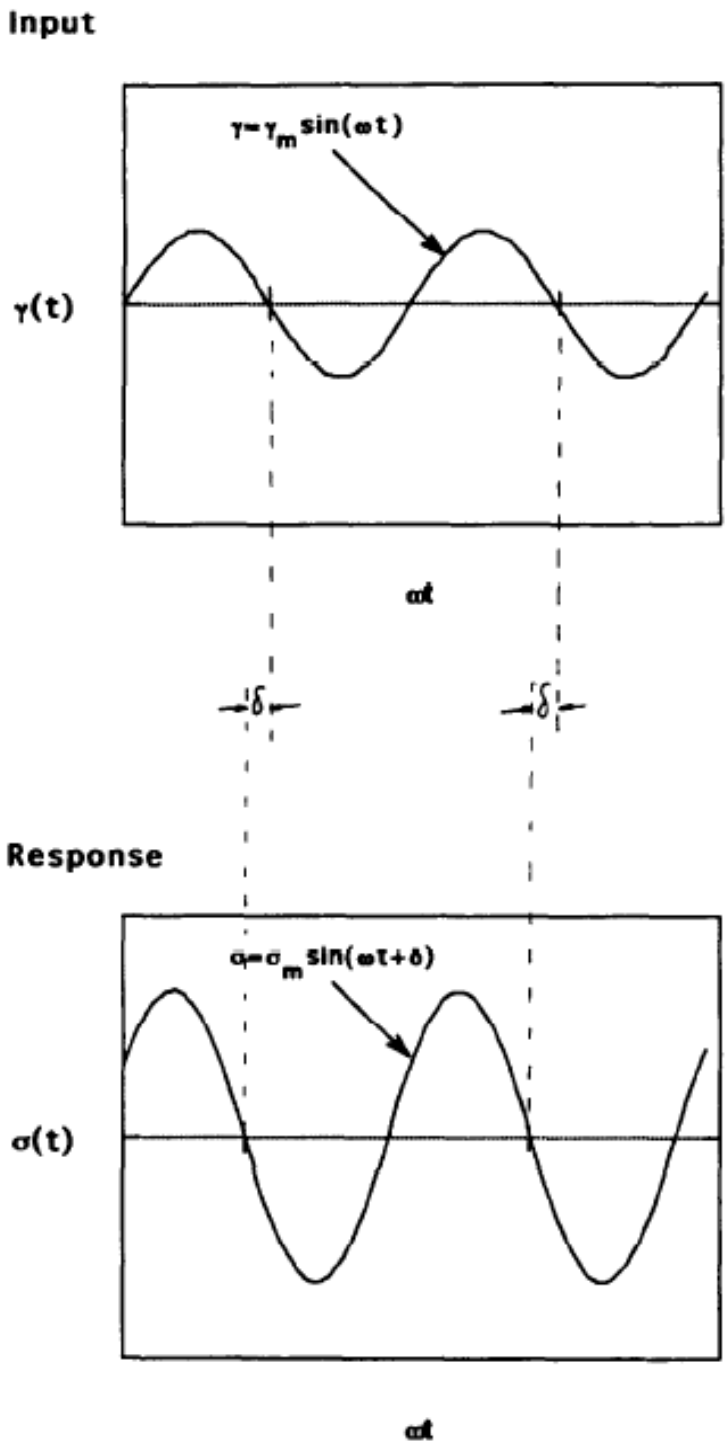


Figure 10. Viscolelastic Response of an Elastic Material.

Likewise, similar definitions can be applied for testing in the normal mode for the complex modulus and complex compliance.

In the dynamic mode of testing, if the strain is a complex oscillatory function of time with maximum amplitude γ_m and frequency ω, then

$$\gamma^* = \gamma_m \exp(i\omega t) \tag{33}$$

By analogy with the Maxwellian element of viscoelasticity is

$$\frac{d\gamma^*}{dt} = \frac{1}{G}\frac{d\sigma^*}{dt} + \frac{\sigma^*}{G\theta} \tag{34}$$

where θ is a characteristic time constant and is equal to η/G.

Here, G has no physical meaning related to the dynamics of the polymer, but describes the modulus of the analogue. The same is true of η, which has no physical meaning related to the viscosity dynamics of the polymer, but again describes the viscosity of the analogue. Taking the derivative of the shear strain in Equation 33 and substituting into Equation 34, we can then solve the linear differential equation and the solution is given below [Anseth 1996]

$$\frac{\sigma^*}{\gamma^*} = \frac{G\omega^2\theta^2}{1+\omega^2\theta^2} + i\frac{\omega\theta G}{1+\omega^2\theta^2} \tag{35}$$

Or

$$\sigma^* = \sigma_m \exp(i\omega t + \delta) \tag{36}$$

where the measured response of the shear stress is then shifted by a phase angle delta with a maximum amplitude σ_m.

Thus, by comparison of Equations 32 and 35

$$G' = \frac{G\omega^2\theta^2}{1+\omega^2\theta^2} \tag{37}$$

and

$$G'' = \frac{G\omega\theta}{1+\omega^2\theta^2} \tag{38}$$

If we define the ratio of the loss to the storage modulus as the damping or dissipation factor, then

$$\frac{G''}{G'} = \frac{1}{\omega\theta} = \tan\delta \tag{39}$$

The damping factor, tan delta, measures the ratio of the energy dissipated as heat to the maximum energy stored in the material during one cycle of oscillation. Representative plots of the storage, loss, and damping factor are shown in Figure 11.

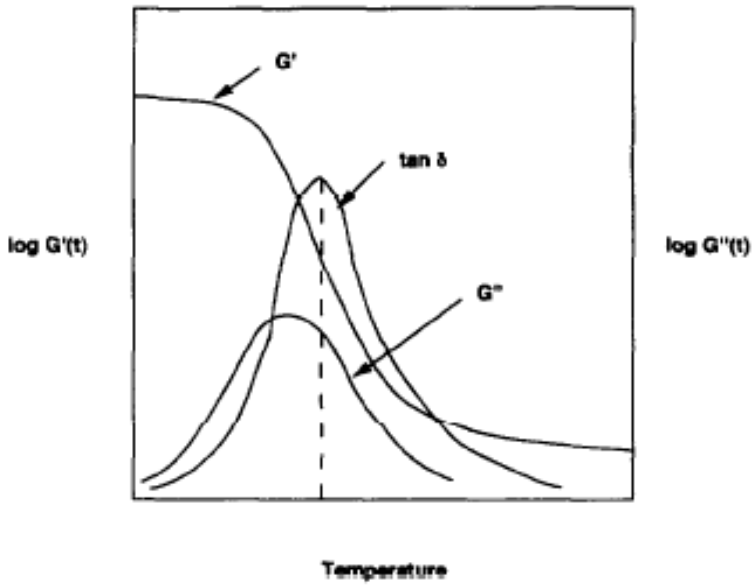


Figure 11. Loss and Storage Modulus as a Function of Temperature.

At low times or temperatures, the polymer is glassy and the storage modulus, G', is in an unrelaxed state. As the temperature is increased, the viscoelastic nature of the materials is seen and a transition region in G' is observed. At still higher temperatures, another plateau in G' occurs as the polymer is now in a relaxed, rubbery state. In contrast, the loss modulus, G'', goes through a maximum as the temperature is increased. G'' is a measure of the viscous contribution to the modulus. The viscous contribution is at a maximum near the inflection point of the transition region in G'. The characteristic shape of the damping factor versus temperature is also shown. This curve provides one of the mechanical definitions for the glass transition temperature, α-relaxation. The glass transition temperature is taken as the temperature at which the maximum in the damping factor curve is observed. Other transitions associated with side chains or branches are also often observed as peaks. The transition associated at the highest temperature is often termed the α-relaxation, while subsequent temperature transitions are labeled as β-relaxations, etc. in order of descending temperature at which the transition occurs. Depending on the homogeneity of the network, the peak associated with the α-relaxation (Tg) can be quite broad [Anseth 1996].

Natural Polymers

Natural polymers offer the advantage of being very similar, often identical, to macromolecular substances which the biological environment is prepared to recognize and to deal with metabolically. The problems of toxicity and stimulation of a chronic inflammatory reaction, as well as lack of recognition by cells, which are frequently provoked by many synthetic polymers, may thereby be suppressed. Furthermore, the similarity to naturally occurring substances introduces the interesting capability of

designing biomaterials that function biologically at the molecular, rather than the macroscopic, level. On the other hand, natural polymers are frequently quite immunogenic. Because they are structurally much more complex than most synthetic polymers, their technological manipulation is quite a bit more elaborate. On balance however these opposing factors have conspired to lead to a substantial number of biomaterials applications in which naturally occurring polymers, or their chemically modified versions, have provided unprecedented solutions.

An intriguing characteristic of natural polymers is their ability to be degraded by naturally occurring enzymes, a virtual guarantee that the implant will eventually be metabolized by physiological mechanisms. This property, at first glance, may appear as a disadvantage since it detracts from the durability of the implant. However, it has been used advantageously in biomaterials applications in which it is desired to deliver a specific function for a temporary period of time, following which the implant is expected to degrade completely and to be disposed of by largely normal metabolic processes. It is possible to control the degradation rate of the implanted polymer by chemical crosslinking or other chemical modifications [Dumitriu 1996].

Many natural polymers form three-dimensional networks. These gels are usually stabilized by interchain interactions and have properties comparable to chemically crosslinked synthetic gels. There are also numerous biomolecules and cell types that have been immobilized on and within hydrogels [Dumitriu 1996].

Two-thirds of the human body consists of water, and almost all known biological reactions occur in water. Water needs a container to act as a medium where chemical reactions can occur: nature invented two kinds of containers- cells and gels. The ability

of polysaccharides to form a network structure, gel, even at low concentrations, constitutes one of their most important functional properties. Recently, there is increased interest in starch gelation in the pharmaceutical field because of the need for three-dimensional matrices with controlled release properties. Gelation is the formation of a three-dimensional network structure and offers an effective means to increase a polymer system's mechanical and chemical stability. Polysaccharide mixtures occur naturally, and binary carbohydrate gels may be used as models for complex cellular structures and have been studied as models for the recognition step in certain host-pathogen interactions [Dumitriu 1996].

There are many structures that can be formed when two polysaccharides are mixed together and gelled. These are dependent on the nature of the components, the rate and extent of demixing, and mechanism of gelation. The simplest structure is obtained when component one forms a network and component two is just contained in component one. At least three types of gel structure can occur if both polysaccharides contribute to the network. One, the gel is an interpenetrating network where both polysaccharides associate independently to form separate networks but interlace with each other. Two, a phase-separated network occurs if some degree of demixing occurs prior to gelation and the two networks are spatially separated. Three, a coupled network is formed if the two networks chemically bond to each other during gelation [Dumitriu 1996].

The attachment of biologically active compounds to synthetic and natural polymers has been investigated as a means of improving the efficacy of drug controlled release devices through a constant but prolonged release of bioactive compounds with minimum side effects. The controlled release of the bioactive compounds, which are

covalently coupled to the polymeric carrier via hydrolysable linkages, can be achieved by hydrolytic or enzymatic cleavage of the linking bonds [Dumitriu 1996].

One of the main challenges in designing drug controlled release devices is to construct biocompatible carriers that are easily metabolized and eliminated, that do not adversely affect normal biological functions inside the human body, and are inexpensive for reducing health care costs. A class of biomaterials that could be a potential candidate as the drug carrier is natural polymers, such as polysaccharides [Dumitriu 1996].

Naturally occurring polysaccharides are one of the most abundant and diverse families of biopolymers, and are widely used in various fields. Recently, considerable attention been focused on chemical modification of polysaccharides to be used as drug carriers for controlled release systems. The repeating unit of polysaccharides may contain hydroxyl, carboxyl, amino, or sulfate functional groups, which provide the sites for the chemical modification of polysaccharides. Starch, long-chain polymers of D-glucose, is one of the most frequently used polysaccharides in the drug delivery system because of its biocompatibility, biodegradability, nontoxicity, and abundant sources [Dumitriu 1996].

Lenaerts et al. demonstrated that crosslinked high amylase starches have been used as excipients for the formation of controlled release solid dosage forms for the oral delivery of drugs. Corn starch was highly crosslinked by using epichlorohydrin as a crosslinking agent. Water transport kinetics and dimensional changes were studied in matrices placed in water at 37C by an image analysis technique. The studies showed that a gel was formed very quickly at the polymer surface and water continually diffused into the core. This process went on for several hours until the core turned into a gel and

equilibrium swelling was reached. In vivo results with different drugs showed that high amylase crosslinked starches were highly versatile, had no food or drug interaction.

Remon et al. reported the production and characterization of thermally modified starch which might be used as hydrophilic matrices for oral drug delivery systems. Three native starches having a variety of amylase/amylopectin contents were pregelatinized by extrusion, drumdrying, and a controlled pregelatinization-spray-drying technique in order to produce cold-water-swellable starches. Partial pregelatinized starch could only be produced by the controlled pregelatinization-spray-drying technique. As regards the application as hydrogel matrices for controlled release tablets, promising results were seen for the extruded drum-dried starches containing medium to high amount of amylopectin.

Amylose and Amylopectin are the two components of naturally occurring starch. Amylose is a relatively linear homopolymer of glucose with a low molecular weight. Amylopectin is a highly branched homopolymer of glucose with a relatively high molecular weight. Starch in both native and modified forms has been studied extensively over the past 50 years. Crosslinked starch and starch networks have both the required biodegradability and a reasonable chemical and mechanical stability. In its original form starch occurs as a granule made up of several layers of alternating crystalline and amorphous layers. The regions of long-range crystallinity seem to involve crystallization of the amylopectin component of starch while the amylose component represents the amorphous phase of starch granules [Dumitriu 1996].

A variety of physical techniques such as x-ray diffraction (XRD), small angle neutron scattering (SANS), small angle x-ray scattering (SAXS), and electron

microscopy (EM) have provided information on the nature of the amylopectin crystallites. SANS, SAXS, and EM have shown periodicity occurring at 10nm which is thought to be the repeat distance between amorphous and crystalline regions of amylopectin [Dumitriu 1996].

Opaque gels are obtained upon cooling of concentrated starch aqueous dispersions. At this stage, elementary junction zones are locally established between the macromolecules. These adopt locally a left-handed, parallel-stranded double helical conformation. On aging, starch gels develop a B-type crystallinity from an aggregation process resulting in parallel arrays of the elementary junction zones. A new three-dimensional structure of B-starch is proposed in which the unit cell contains 12 glucose residues located in two left-handed, parallel-stranded double helices packed in parallel register, with 36 water molecules located between these helices. It is now well established that amylose gels are poorly crystalline structures composed largely of an amorphous fraction [Dumitriu 1996].

Leloup et al. have investigated the structural characteristics of amylose gels (2%-8% w/vol) at different scales of organization. Native gels have been studied by electron microscopy, mild acid hydrolysis, differential scanning calorimetry, and size-exclusion chromatography. Amylose gels exhibit a macroporous structure, mesh size 100-1000nm, containing filaments 20$\pm$10 nm wide. These filaments result from the association of segments of amylose chains which were oriented obliquely to the filament axis. These amylose fragments are partially organized in a B-type crystalline array. Upon acid or enzymatic treatments, the amorphous segments of amylose chains are hydrolyzed [Dumitriu 1996].

Amylopectin can have a very high molecular weight as large as 10^8 though measuring the molecular weight has been difficult due to the aggregation of the chains of the polymer in solution which can lead to an over-estimation of the size of the molecules. [Durrani and Donald 1995]. It has been recently observed that at sufficiently high concentrations solutions of amylopectin in water could gel when cooled to below room temperature.

Even though there are many investigations of biopolymer gels, there are relatively few studies of amylopectin. Amylopectin is one of the two main components of starch. Amylopectin consists of D-glucopyranose monomers which are connected either through alpha-1-4 or alpha 1-6 glycosidic bonds [Durrani and Donald 1995]. The 1-4 bonds produce the linear backbone of the chain and the 1-6 bonds are the branch points off the main chain backbone. Amylopectin is a high molecular weight natural polymer with non-random branches. The branching of the polymer is extensive and responsible for the molecule's large hydrodynamic volume and ability to gel at concentrations of 3% and above in aqueous solution. The branching is non-random, and the architecture of the molecule is debated because of the influence of steric hindrance and the placement of inner and outer chains. The most accurate molecular structure seems to be described by the cascade type branching theory with the amylopectin branches arranging themselves in clusters of tiered branches as illustrated in Figure 12 [Durrani and Donald 1995].

Gelation is the crosslinking of many polymer chains into progressively larger branched polymers to an extent ultimately producing a single molecule spanning the entire system. Starch gelatinization is the process by which solubilization, hydration, and swelling of starch molecules occurs. As the dispersed starch is heated in solution, the

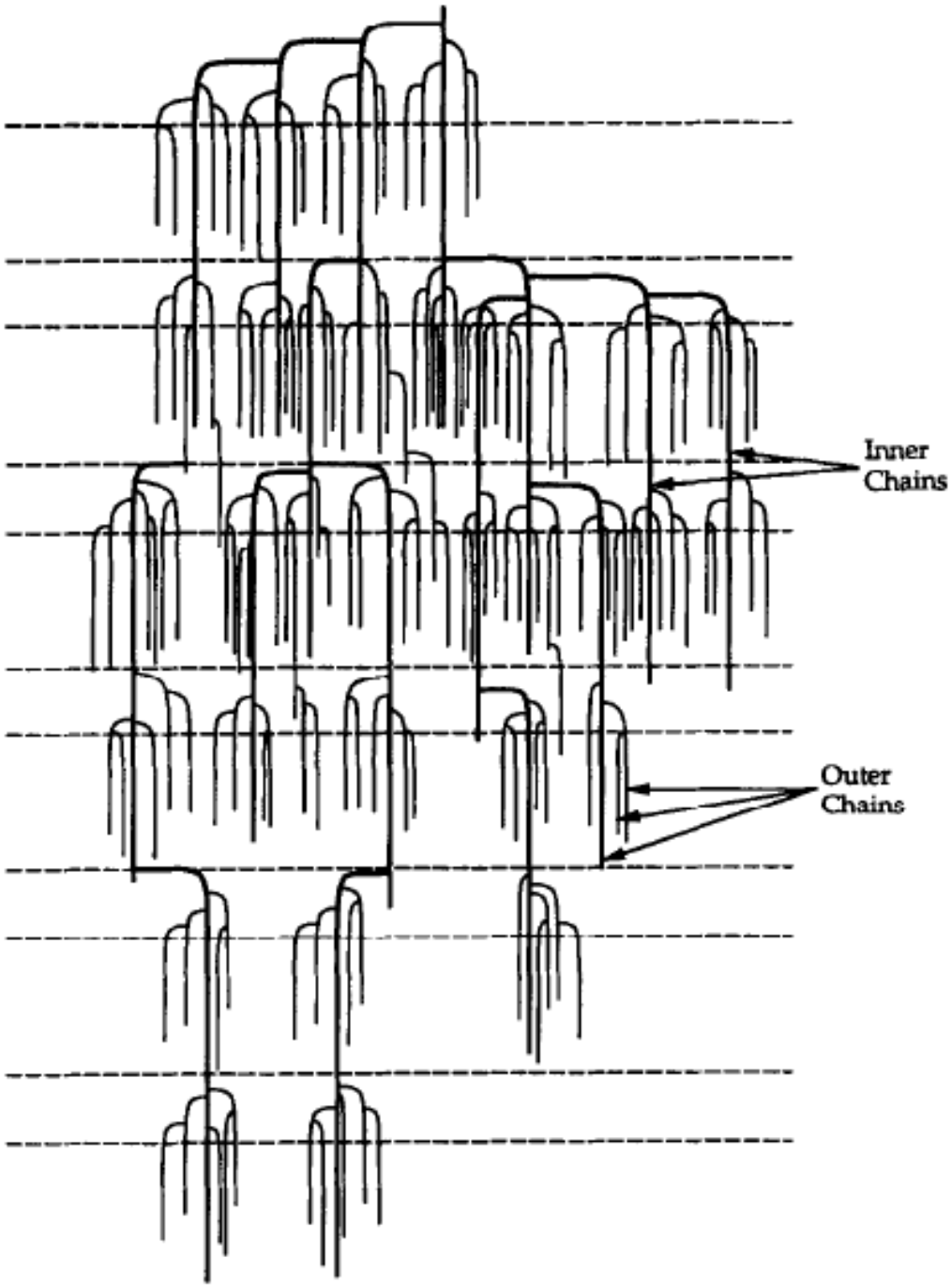


Figure 12. Schematin of Amylopectin Structure.

randomly coiled chains uncoil and become hydrolyzed then recoil upon cooling creating physical entanglements among neighboring chains [Whistler 1984].

Hydrogen bonding is the second most important mechanism of starch gelation next to physical entanglement, and the solvent properties of water are solely responsible for the hydrogen bonding of the starch gels [Morawetz 1965]. In the Figure 13, oxygen is surrounded tetrahedrally by four hydrogen atoms- two with which it forms a covalent bond, and two belonging to neighboring water molecules to which it is hydrogen bonded.

A hydrogen bond can form between two neighboring water molecules only if a hydrogen lies close to the line connecting their oxygen atoms. This prevents the molecules from packing as efficiently as they would if their interactions were due solely to dispersion forces and dipole-dipole interactions [Morawetz 1965]. During the gelation of starch in water hydrogen bonding occurs around the perimeter of the starch helices as well as the within the alpha helix. The alpha helix is capable of accommodating as many as 32 interstitial water molecules per turn of the alpha helix [Morawetz 1965]. Previous studies have shown that gelation of an amylopectin solution is accompanied by the development of crystallinity, deduced from the growth of diffraction peaks in x-ray diffraction patterns. It is thought that an amylopectin gel is a network which is formed by junction points of ordered crystalline regions within the system of the amylopectin molecules. The gels are white and opaque, indicating that they consist of phase separated regions on a length scale of the order of the wavelength of visible light [Durrani and Donald 1995].

The phenomenon of polymer immiscibility is well known and arises as a result of the generally unfavorable interactions between polymer species. Even a small positive free energy of interaction between different polymer species can result in limited

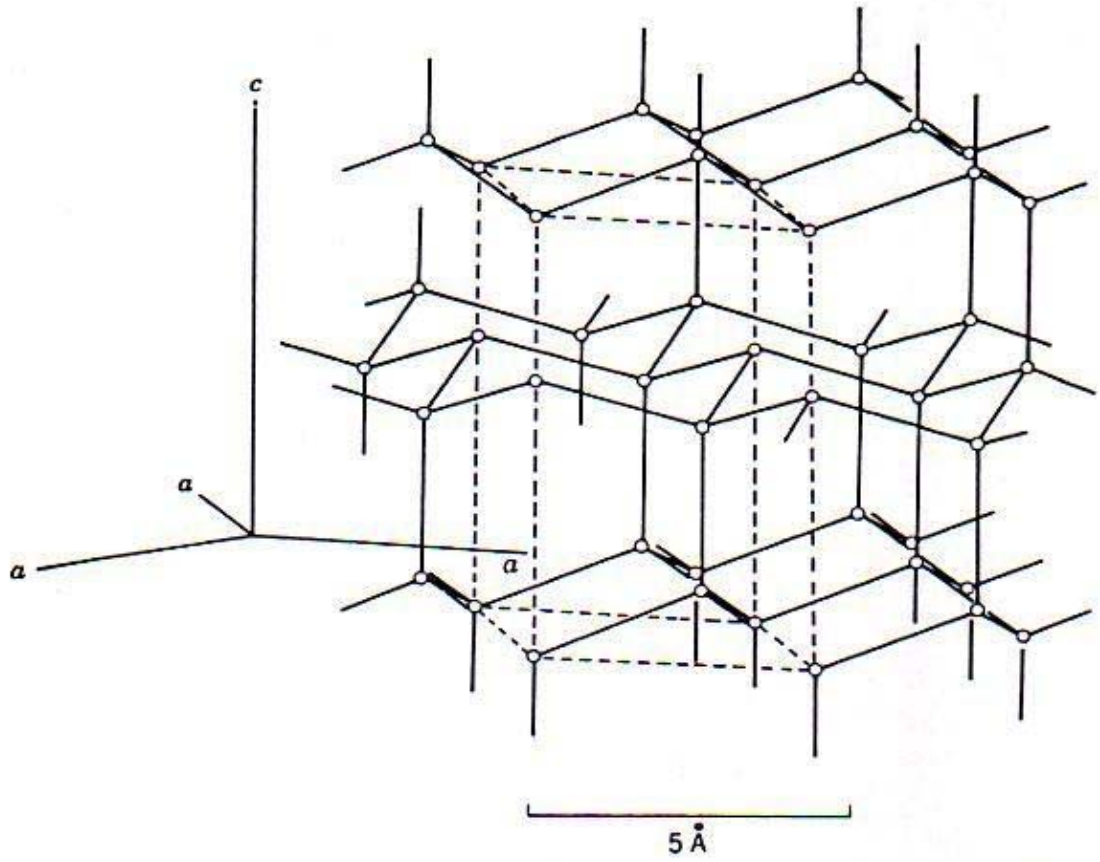


Figure 13. Schematic of Hydrogel Bonds in Water Molecules.

miscibility due to the small entropy gain on mixing these high-molecular weight species. The miscibility of polymers in solution decreases with increasing polymer concentration and is rare at high concentrations. If the segregation factor is strong, demixing is predicted when the polymer chains start to become entangled about the coil-overlap threshold. The phase behavior of these ternary systems polymer1 +polymer 2 + solvent is often strongly affected by polymer-solvent interactions. Immiscibility is generally increased when the affinity of one polymer for the solvent is significantly different from that of the other. Biopolymers also exhibit immiscibility, with perhaps the best known example being the gelatin-gum Arabic-water system. Compared to synthetic polymer mixtures, there is relatively little information on the phase behavior of polysaccharide mixtures.

Starch occurs as a mixture of amylose and amylopectin which are organized into a semi-crystalline granule. The behavior of aqueous solutions of starch is of interest because starch is processed by heating in the presence of water. Whereas amylose is essentially a (1-4)alpha-D-glucan, amylopectin is a (1-4)alpha –D-glucan with an average of one in every 20-25 residues branched at position 6. As these polymers are so chemically similar, it might be expected that immiscibility would only be observed at very high concentrations. However, even moderately concentrated aqueous solutions of amylose and amylopectin exhibit immiscibility [Kalichevsky 1987].

According to Kalichevsky's work using mixtures of amylose and amylopectin of varying concentrations from pure amylose to pure amylopectin in a 10% total aqueous solution, the binodal phase diagram is not symmetric but is shifted towards the amylose-rich phase. This is consistent with the behavior predicted for a mixture containing

polymers of unequal molecular weight, the bimodal being displaced towards the polymer of lower molecular weight. The tie lines between phases at equilibrium with each other slope up to the amylose rich phase, indicating that this phase has a higher affinity for the water solvent [Kalichevsky 1987].

The investigation of the phase behavior of amylose and amylopectin, using amylose of different molecular weights and structures as well as different temperatures (70C-90C), shows that these factors do not strongly affect the unfavorable interaction between these polysaccharides which gives rise to the phase separation. It is noted that the differences in molecular weight between the amylose samples used are small relative to the large differences between amylose and amylopectin. From the observation that phase separation only occurs at concentrations well above C*, minimum polymer concentration to induce phase separation, it is apparent that the segregation factor is not very strong. It has been observed that immiscibility becomes greater with increasing molecular weight, so the high molecular weights of these polysaccharides, especially the amylopectin, encourage phase separation. The incompatibility of the linear and branched polymers of starch in aqueous solution has important implications. Incompatibility may also affect the types of interactions which can occur and should be considered as part of the gelatinization behavior of starch on heating in water [Kalichevsky 1987].

Crosslinking of Starch

Chemical crosslinking is defined as a direct reaction of a linear or branched polymer with at least one difunctional, small molecular weight, crosslinking agent. The purpose of this agent is to link two larger molecular weight chains through its di- or multifunctional reactive chemical groups [Ratner 2004]. Crosslinking agents can be added to

a polymer solution after the polymer has been produced, and the reactive species of the agents will react with the polymer chains forming a chemically crosslinked system. (See Figure 14.)

Copolymerization crosslinking is a reaction between a solution of one or more types of monomers including one multifunctional monomer that is present in relatively small quantities where the polymerization of the polymer and the chemical crosslinking of the system occurs in one step. Another method is the combination of monomer and linear polymeric chains that are crosslinked by means of an interlinking agent [Ratner 2004].

Crosslinking is one of the oldest methods for the chemical modification of polymers. Various degrees of crosslinking can be introduced into polysaccharides for the purpose of generating larger molecular aggregates with enhanced viscosity profiles, or for the preparation of insoluble products with a wide range of swelling characteristics [Dumitriu 1996]. Some of the most common crosslinking agents include bi- and tri- functional reagents, such as epichlorohydrin, bisepoxides, dihalogenated reagents, glutaraldehyde, acetaldehyde, formaldehyde, maleic and oxalic acid, dimethylurea, polyacrolein, diisocyanates, divinyl sulfate, ceric redox systems, and s-triazine. There are also factors to be considered when chemically crosslinking such as the extent of the reaction and the number and character of side reactions. An important parameter often used for identification of the final crosslinked structure is the cross linking ratio, CR, which is defined as the ratio of moles of crosslinking agent to the moles of polymer repeating units [Dumitriu 1996]. For bifunctional crosslinking agents, the number average molecular weight

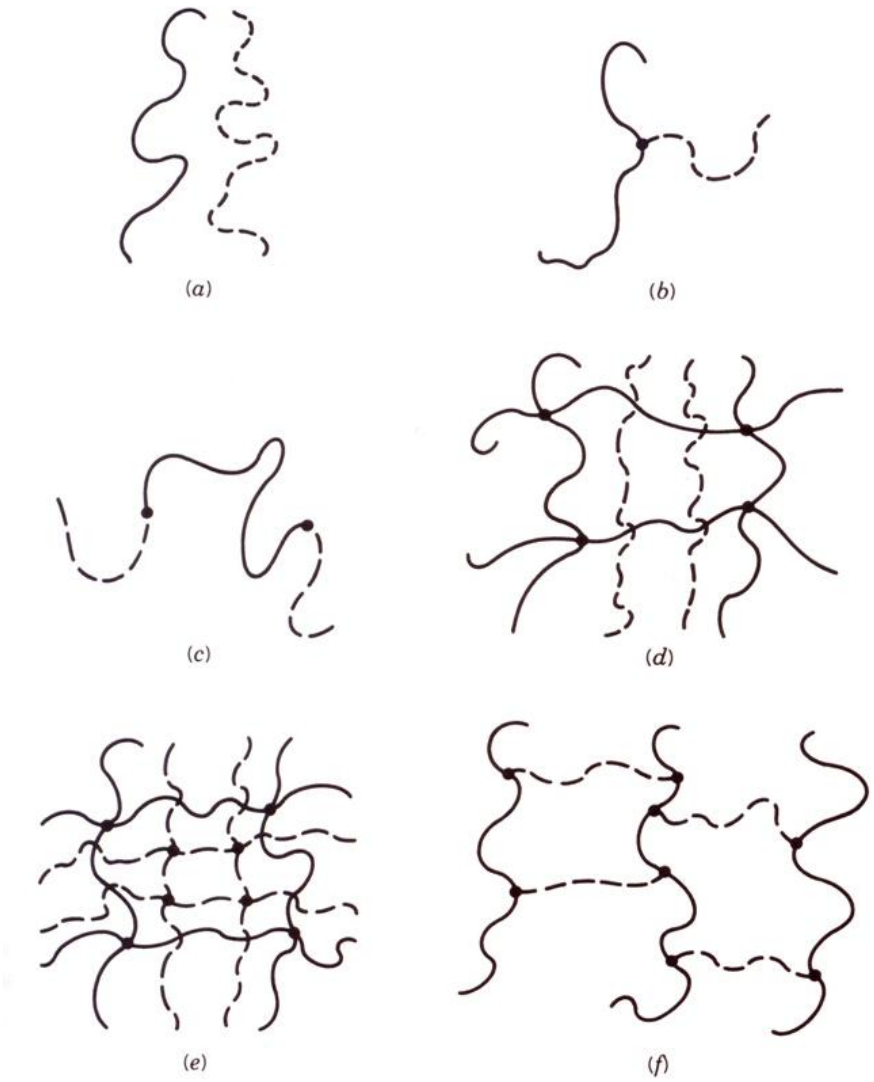


Figure 14. Steps in Formation of a Network of Polymer and Crosslinker.

between crosslinks, M_c, may be determined by the relation

$$M_c = \frac{M_r}{2CR} \qquad (40)$$

where M_r is the molecular weight of the repeating unit.

The addition of a chemical crosslinker is important in order to improve both the integrity of the gel and also the predictability of mechanical properties. Starch gels alone are inconsistent mechanically because of the extent of phase separation, formation of aggregates, and induced crystallinity of the system. One aspect of biopolymer gelation which has been addressed by many authors is the relationship between gel modulus and concentration. This relationship tests network theories which relate the molecular structure of the gel to the most obvious macroscopic aspect of the gel, the mechanical properties [Durrani and Donald 1995].

Crosslinked High Amylose Starch (CLHAS) was introduced a few years ago as the controlled release device, Contramid®. Contramid® is a gel of starch crosslinked with epichlorohydrin. It swells in water to form an elastic gel and the ability to regulate the swelling and thus regulate drug release in aqueous media as a function of crosslinking density makes this hydrogel particularly suitable for pharmaceutical applications. The permeability of solutes across the hydrogel barrier, a dynamic state throughout the polymer device, depends on the texture of the hydrogel. For instance, the texture of the helices of a starch hydrogel will effect the diffusion of solutes differently than a network that is made up of linear chains, and for the same reason polymer hydrophilicity and crystallinity play an important role in drug release. Appropriate resistance of swollen tablets and good control of the drug release were only obtained for CLHAS with

moderate crosslinking degrees. Higher degrees of crosslinking generated a sharp decrease in the release time and under certain conditions can even afford disintegrant properties for CLHAS. The non-monotonic variation of the drug release time with crosslink density is a particular characteristic of the CLHAS matrix that differs from those of other classical polymeric matrices for which increasing the crosslink density leads to longer release times. This behavior of CLHAS was ascribed to the particular structure of the matrix where, in the case of low crosslink density, covalent linkages, interchain hydrogen bonds, and water-promoted hydrogen associations stablilize the network thus controlling the access of water into the matrix. Aspects of the water uptake as a function of crosslink density and its role in the release behavior of CLHAS tablets have been reported.

The native starch granule is heterogeneous both chemically, amylose and amylopectin, and physically, crystalline and amorphous regions. The presence or absence of crystalline order is often a basic underlying starch properties. Depending on their origins, various types of native starches present specific morphologies giving distinctive X-ray powder patterns termed type A, B, or C polymorphs. The sharpness of the X-ray diffraction pattern of starch granules depends on their water content, the B type being more sensitive to hydration than the A-type starch.

For a better understanding of the role of crystallinity in release control, Ispas-Szabo et al studied a series of powders, tablets, and films of high amylose starch with varying degrees of crosslinking. When the crosslink density changes, the morphology of powder, tablet, or film also changes. The variation of X-ray diffractograms as a function of crosslink density for powders and tablets was described. They propose that the

compression of powders leads to molecular rearrangements and possibly to more extended hydrogen association. When the powders are compressed, they believe that the aggregates of the polysaccharide chains come closer together, and their ability to resist an external force is related to the new molecular structure. There is data that crushing strength of CLHAS tablets has been shown to depend on the crosslink density.

The presence of water and its influence on solid carbohydrate structure is often reflected in their spectra. FTIR studies have correlated peaks at 1646 and in the 1300-800 range as a region sensitive to the structure of the polysaccharides. These investigators made films of CLHAS with varying crosslink densities and correlated the spectral shifts with the molecular conformational transitions from B- to V- type helix structure that was later confirmed by X-ray diffraction. The x-ray and FTIR analysis correlated with dissolution kinetics and mechanical hardness of the dry tablets can generate interesting information on the structure-properties relationship in CLHAS matrices.

II. Chemistry

Formation of Crosslinked Network

Starch

Polysaccharides are compounds made up of many hundreds or even thousands of monosaccharide units per molecule. As in disaccharides, these units are held together by glycoside linkages, which can be broken by hydrolysis. Polysaccharides are naturally occurring polymers, which can be considered as derived from aldoses or ketoses by polymerization with loss of water. A polysaccharide has the general formula $(C_6H_{12}O_5)_n$. By far the most important polysaccharides are cellulose and starch. Both are produced in plants from carbon dioxide and water by the process of photosynthesis, and both are made up of D-(+)-glucose units. Cellulose is the chief structural materials of plants, giving the plants rigidity and form. It is probably the most widespread organic material known. Starch makes up the reserve food supply of plants and occurs chiefly in seeds. It is more water-soluble than cellulose, more easily hydrolyzed, and hence more readily digested [Morrison and Boyd 1983].

Starch occurs as granules whose size and shape are characteristic of the plant from which the starch is obtained. When intact, starch granules are insoluble in cold water; if the outer membrane has been broken by grinding, the granules swell in cold water and form a gel. When the intact granule is treated with warm water, a soluble portion of the starch diffuses through the granule wall; in hot water the granules swell to such an extent that they burst [Morrison and Boyd 1983].

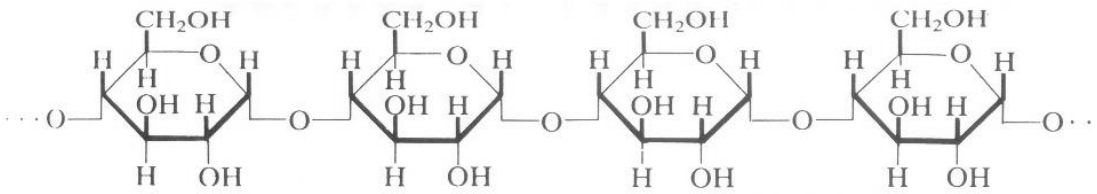


Figure 15. Amylose

In general, starch contains about 20% of a water-soluble fraction called amylose, and 80% of a water-insoluble fraction called amylopectin. These two fractions appear to correspond to different carbohydrates of high molecular weight and formula $(C_6H_{12}O_5)_n$. Upon treatment with acid or under the influence of enzymes, the components of starch are hydrolyzed progressively to dextrin, a mixture of low molecular weight polysaccharides, (+) maltose, and finally to D-(+)-glucose. Both amylose and amylopectin are made up of D-(+)-glucose units, but differ in molecular size and shape as discussed previously.

The only disaccharide that is obtained by the hydrolysis of amylose is (+)-maltose, and D-(+)-glucose is the only monosaccharide. To account for this, it has been proposed that amylose is made up of chains of many D-(+)-glucose units, each unit joined by an alpha-glycosidic linkage to C-4 of the next one [Morrison and Boyd 1983]. Amylose, as shown in Figure 15, is made up of long chains each containing 1000 or more D-glucose units joined together by alpha-linkages and there is little or no branching of the chain.

Like amylose, amylopectin is made up of chains of D-glucose units, each unit joined by an alpha-glycoside linkage to C-4 of the next one as shown in Figure 16. However, its structure is more complex than that of amylose. Molecular weights

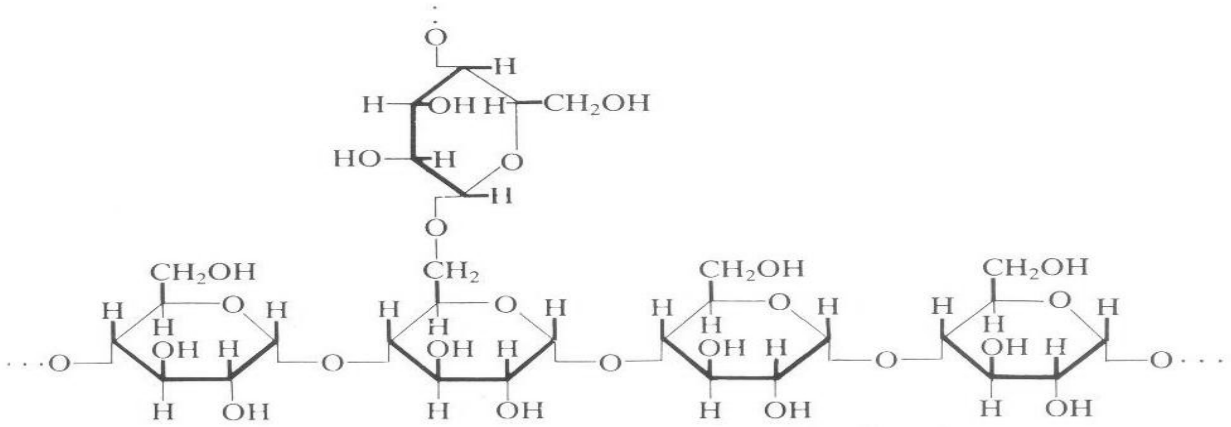


Figure 16. Amylopectin.

determined by physical methods show that there are up to a million D-glucose units per molecule. Amylopectin has a highly branched structure consisting of several hundred short chains of about 20-25 D-glucose units each. One end of each of these chains is joined through a C-1 to a C-6 on the next chain [Morrison and Boyd 1983].

Ester Linkage

An ester linkage is the product of a condensation reaction of an acid and an alcohol. Condensation is a chemical reaction in which two molecules join together and in the process eliminate a smaller molecule. In the reaction of the acid and the alcohol the –OH groups of each react and eliminate a water molecule creating a –COO-C- bond. Ester linkages degrade hydrolytically. The most common esters are formed from carboxylic acids [Morrison and Boyd 1983].

Dicarboxylic acids have two reactive –COOH ends. Because of the two charged ends, the reactivity of one end may affect the other resulting in different pKa values of each carboxyl group. This however is dependent on the length of the molecule, the

shorter the molecule the larger the difference in the reactivities of the ends. The chemical properties of di-acids are usually comparable to monocarboxylic acids [Morrison and Boyd 1983].

Glucaric Acid

D-Glucaric Acid is a dicarboxylic acid derived from glucose [Morrison and Boyd 1983]. Figure 17 lists various glucose derivatives including glucaric acid and sorbitol.

D-glucaric acid is thought to take one of four conformations when dissolved in aqueous solution. All of these conformations seem to be in dynamic equilibrium with each other. D-glucaric acid has a linear conformation with a carboxyl group at each end. Two of the other conformations are a 1,4 lactone and a 6,3 lactone. The fourth conformation is that of a dilactone.

D-glucaric Acid, D-glucaro-1,4-lactone, D-glucaro-6,3-lactone, and d-glucaro-1,4:6,3 dilactone are compounds of significant biological interest [Horton 1982]. (See Figure 18.) They are normal metabolites involved in the metabolism of d-glucuronic acid in mammals; d-glucaro 1,4, lactone is a powerful, competitive and highly specific inhibitor of beta-d-glucosiduronase; and the dilactone is a precursor of the 1,4 lactone. It is thought that the production of the 1,4 lactone from d-glucuronic acid can modify beta-d-glucosiduronase in the body. The 1,4 lactone has been considered as a potential drug for treating diseases in which it is suspected that there may be a disorder of d-glucuronic acid metabolism [Horton 1982]. The 1,4 lactone and the dilactone have been successfully tested in the treatment of rheumatoid arthritis. These compounds also have anticoagulant properties and show some promise in lysosome stabilizing activity.

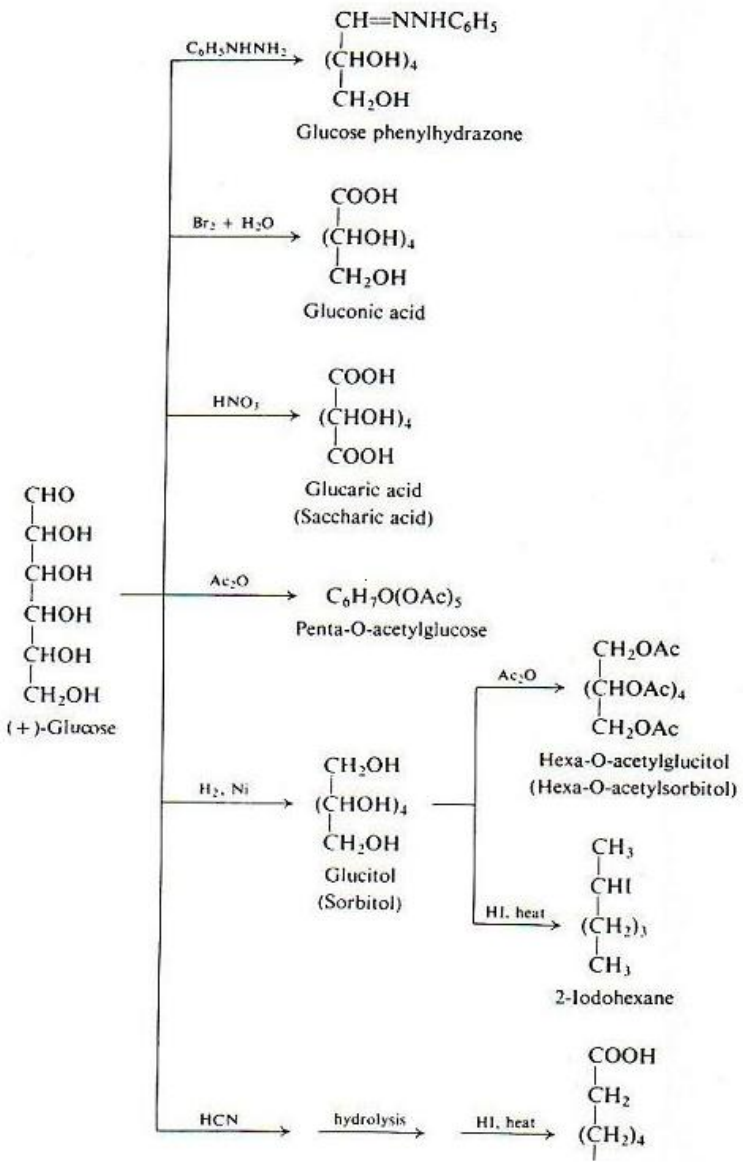


Figure 17. Derivatives of Glucose.

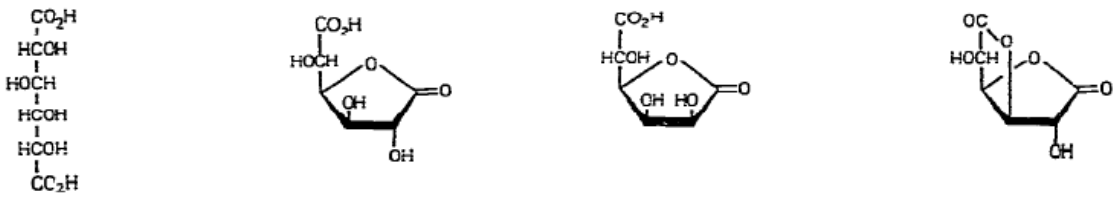


Figure 18. Conformations of D-glucaric Acid in Aqueous Solution.

III. Materials and Methods

Materials

Amylose, amylopectin, and D-saccharic acid potassium salt were all purchased from Sigma Aldrich (St. Louis, MO, USA).

Synthesis

The starch gel was chemically crosslinked by condensation of the –OH groups of both amylose and amylopectin with the acid ends of the glucaric acid to form two ester linkages. The crosslinked starch gels were synthesized by first dissolving glucaric acid potassium salt in deionized water. Amylopectin dissolves only in cold water solutions while amylose dissolves only in hot water solutions, so each glucaric acid solution was allowed to cool to room temperature before adding the amylopectin powder and then the solution was heated before adding the amylose. All solutions were constantly stirred to prevent sedimentation. The amylose aggregates instantly if the water temperature is too low limiting the diffusion of water into the system, but the amylopectin will gel when the solution reaches approximately 75C. The amylopectin glucaric acid solution was heated to approximately 55C before the amylose powder is incorporated into the solution. The crosslinked starch solution was then heated until the viscosity of the system increases indicating the formation of the gel. The gel was then removed from the heat source and allowed to cool to room temperature. The synthesized samples are white, opaque, highly viscous, rubber-like gels.

Characterization of Crosslinked Gel

RAMAN

Raman Spectroscopy defines chemical bonds of a sample by taking advantage of the polarizability of the compound within the sample. Raman was performed at room temperature on freeze dried samples.

Rheology

Rheology is the study of the deformation and flow of matter. These properties are based on the micro- or nanostructure of the material including the molecular weight and structure of polymers in solution or the particle size distribution in a solid suspension. To measure the mechanical properties, a viscous solution is placed between the parallel plates and a pressure transducer measures changes in torque as one plate is turned while the other plate is held stationary [Morrison 2001]. The force required to turn the plate is converted into mechanical data of the material, usually the elastic modulus. For these experiments, the samples were prepared before testing and the gels were placed between the plates and compressed to 1mm. The edges were coated with mineral oil to prevent water loss. All frequency sweep tests were performed at constant temperature, constant 0.1% strain, and within a frequency range of 0.1-100 radians per second.

Degradation

For the degradation experiments, the gel samples were placed in vials of the same culture media used in the cell study experiments described later, they were observed at room temperature over a period of several days, and the pH change of each solution was

checked periodically to observe the amount of dissociated di-acid that had been released from the system.

Cell Studies

Preliminary studies of the effects of glucaric acid and glucaric acid crosslinked starch gels are complete. These studies were performed in *in vitro* cultures of the U2OS cell line. The U2OS cell line are human osteosarcoma cells. Two types of experiments were performed: cell proliferation assay and colony formation assay. The growth assay is to test the ability of the carcinoma cells to reproduce and multiply in the presence of the gel. The colony formation test is to prove that the carcinoma cells are mobile, and that the glucaric acid drug is not preventing the cells from forming growth colonies or in a sense recruiting surrounding carcinomas to form tumors.

The cells were plated and allowed to rest for 24 hours before introduction of the crosslinked gel. The gel was prepared in the sterile environment of the tissue culture lab. The gel was incorporated into the cell culture media in swollen form.

Colony Forming Unit Assay (CFU):

Cell lines were removed from liquid nitrogen and seeded onto 60 mm plates with media (DMEM, 15% FBS, 10 units of PenStrep) and then passage on a three-day/four-day cycle. These cells were then split and 1,000 cells were seeded onto small plates. Varying concentrations of crosslinker were added to the media, and the cultures were allowed to grow for a period of 8 days. After this growth period, the media was removed from each plate, rinsed with PBS, and 100% methanol was added to each plate to fix the cells for nine minutes. After fixing the cells, Giemsa stain (1.25 ml Giemsa stain, 1.5 ml

100% Methanol, QS to 50 ml) was added to cover the cells and allowed to incubate for 9 minutes. After this incubation, the stain was removed and each plate was rinsed twice with distilled water to remove any excess stain remaining in the plate.

Cell Proliferation Assay:

Cell lines were removed from liquid nitrogen storage and plated on 60 mm culture plates in media (DMEM, 15% FBS, 10 units of PenStrep). At 90% confluence, cell were trypsinized, and re-plated, onto a 100 mm plate, and allowed to grow to 80% confluence. Once confluent, these plates are then trypsinized and counted (to count the cells, 50 µl of the collected cell suspension was added to 100 µl of PBS/EDTA and from that mixture 50 µl of the solution was placed onto a hemocytometer and the four blocks counted, divided by four and multiplied first by the dilution factor and then by 10,000 to get the number of cells per milliliter of cell suspension) and 250,000 cells are seeded onto culture plates and allowed to grow for one day. On day two the crosslinked gel was added to the media. The cells were counted daily for a period of 5 days after the addition of the gel. Various crosslink concentrations were tested.

IV. Results and Discussion

Sample Nomenclature

Many different gels were investigated including amylopectin, amylose, composites of these two, and crosslinked composites with varying glucaric acid concentrations. In the following data amylose is abbreviated as S, amylopectin as P, and glucaric acid as GA. Numbers associated with these abbreviations are concentrations (wt/v %) of the components. A list of sample nomenclature is presented in Table 2. The gels were made in approximately 10mL batches so that multiple parallel plate experiments could be performed on the same sample.

Raman Spectroscopy

The formation of the ester linkage due to the condensation reaction of the carboxylic groups of the glucaric acid with the hydroxyl groups of the starch, amylose and amylopectin was followed using Raman Spectroscopy. The Raman spectra shows the presence of ester linkages upon addition of glucaric acid.

Figure 19 tabulates literature peak ranges for specific chemical bonds in comparison with the peaks measured from the glucaric acid, the crosslinked gel, and the starch samples. From this data, it is confirmed that ester linkages are present at 875, 975, and 1040 and shifts occur at 289, 406,550,830, 935, 976, 1055, 1220, 1265, 1445 with the addition of glucaric acid. The presence of the ester linkage in the Raman Spectra of the crosslinked gel is conclusive proof that the di-carboxylic acid has chemically bonded to the starch.

Table 2. Sample Nomenclature

Abbreviation	Definition
5S_1 5S_2 5S_3	5% (wt/v) Amylose Gel in water. 3 Runs from the same sample.
10S_1 10S_2 10S_3	10% (wt/v) Amylose Gels in water. 3 Runs from the same sample
20P_1 20P_2 20P_3	20% (wt/v) Amylopectin Gel in water. 3 Runs from the same sample.
5S5P_1 5S5P_2 5S5P_3	5% (wt/v) Amylose with 5% (wt/v) Amylopectin Gel in water. 3 Runs from the same sample.
5S10GA_1 5S10GA_2 5S10GA_3	5% (wt/v) Amylose crosslinked with 10% (wt/v) Glucaric Acid Gel in water. 3 Runs from the same sample.
20P10GA_1 20P10GA_2 20P10GA_3	20% (wt/v) Amylopectin crosslinked with 10% (wt/v) Glucaric Acid Gel in water. 3 Runs of the same sample.
5S5P5GA_1 5S5P5GA_2 5S5P5GA_3	5% (wt/v) Amylose with 5% (wt/v) Amylopectin crosslinked with 5% (wt/v) Glucaric Acid Gel in water. 3 Runs from the same sample.

			Carbohydrates		Di-Carboxylic Acid		Glucaric Acid	Amygel	Starch
					480-680		435	425	445
					out of plane			470	470
					CO def		520	525	
							556	550	550
							624	630	
								650	650
					875-870			675	
	800-970		930 polysaccharide		O-H...O	895-855	940	935	935
600-1300	Esters				out of plane def	cyclic 5 mem	960	965	
Aliphatic						ester	984	975	
chains								1040	
							1050	1050	
				953-1180		980-1135	1065	1065	1065
				sac		CA anhydride		1080	1080
			1160-1000	bands		ester		1090	1090
			C-O str	C-C		6 member		1125	1130
	1060-1150			C-O		ring	1220	1220	
	Esters			C-H	1210-1320	acyclic		1265	1255
					C-O str	cyclic	1265		
		1100-1300						1330	1340
		Esters		1210-1310		1180-1345		1350	
				cyclic	1395-1440	long chain			1375
			1460-1200	5 membered	C-O str	aliphatic		1405	1400
			CH, OH str	ester	O-H def	carboxylic acid	1455	1455	
							1470	1470	1470
1450, 1500								1655	
Aromatics					1625-1710		1687		
					C=O str				

Figure 19. Raman Peaks Comparing Crosslinked Starch to Uncrosslinked Starch.

Rheology

The changes in viscosity and modulus as a function of frequency for amylose, amylopectin, and crosslinked gels are presented in Figure 19. The rheological data shows that the viscosity of the gels ranges from 1000 Pa to 100,000Pa at 0.1 (1/s) frequency depending on monomer components and concentrations.

The storage modulus determined from the parallel plate rheological data shows conclusive evidence of increase for starch gels synthesized with the addition of glucaric acid as a chemical crosslinker. Although not shown here, increasing concentrations of chemical crosslinker have a direct effect on storage modulus. However, this data is affected by the inhomogeneity of starch gels. There is measurable scatter in gel storage modulus even within the same sample because the gels are inhomogeneous due to the phase separation behavior of the amylose and amylopectin components. The overall trend illustrated in Figure 20 shows amylopectin gels with the lowest modulus, composite amylose and amylopectin gels in the middle, and amylose gels with the highest modulus.

There were significant increases in modulus of crosslinked gels versus uncrosslinked gels as shown in Figures 21-25 The storage modulus of gels comparing single component, composite, and crosslinked composite also shows increases in modulus with addition of the glucaric acid chemical crosslinker. Figure 24 is a plot of the individual runs and Figure 25 is a plot of the average values.

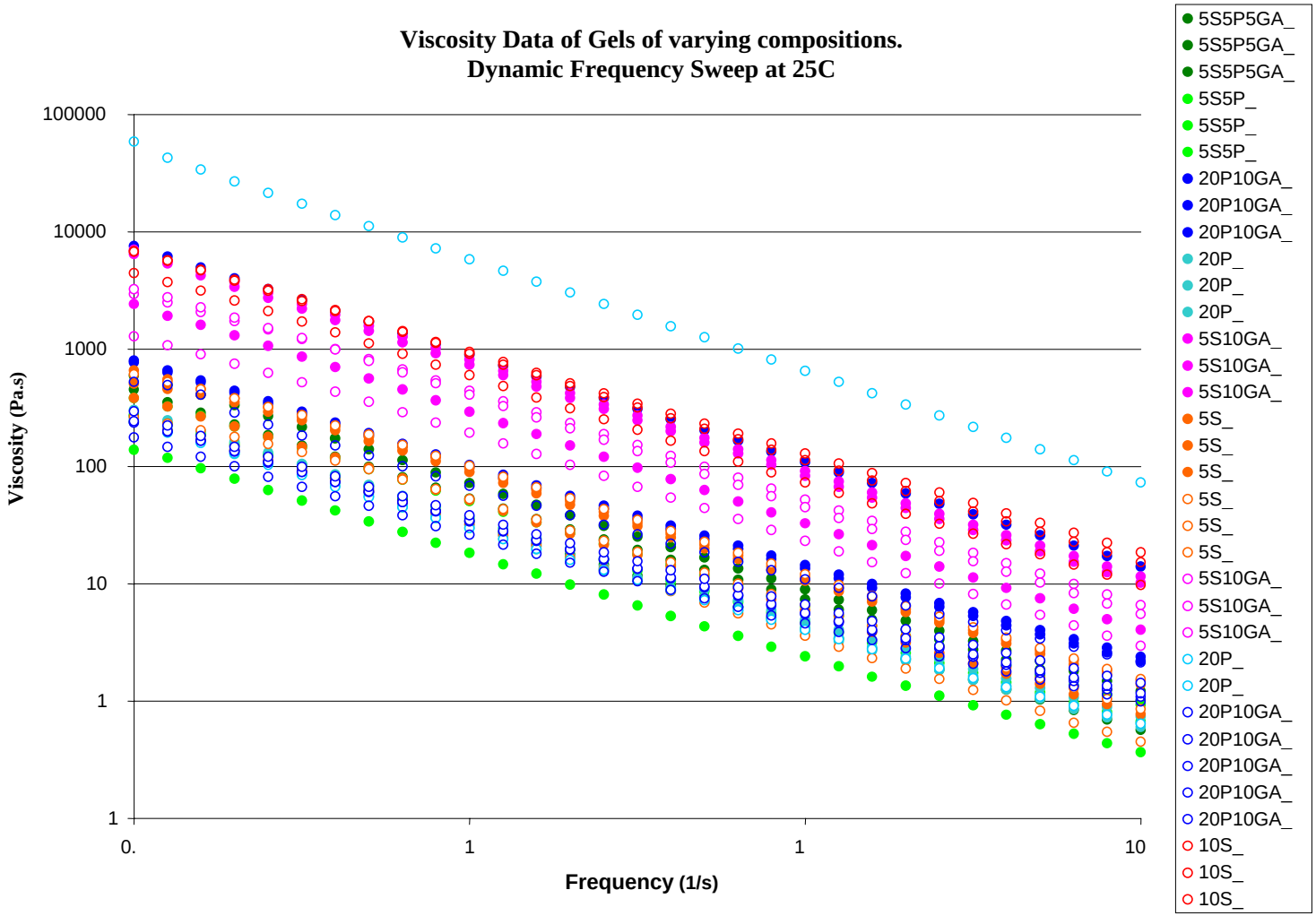


Figure 20. Viscosity Data of Gels of Varying Compositions.

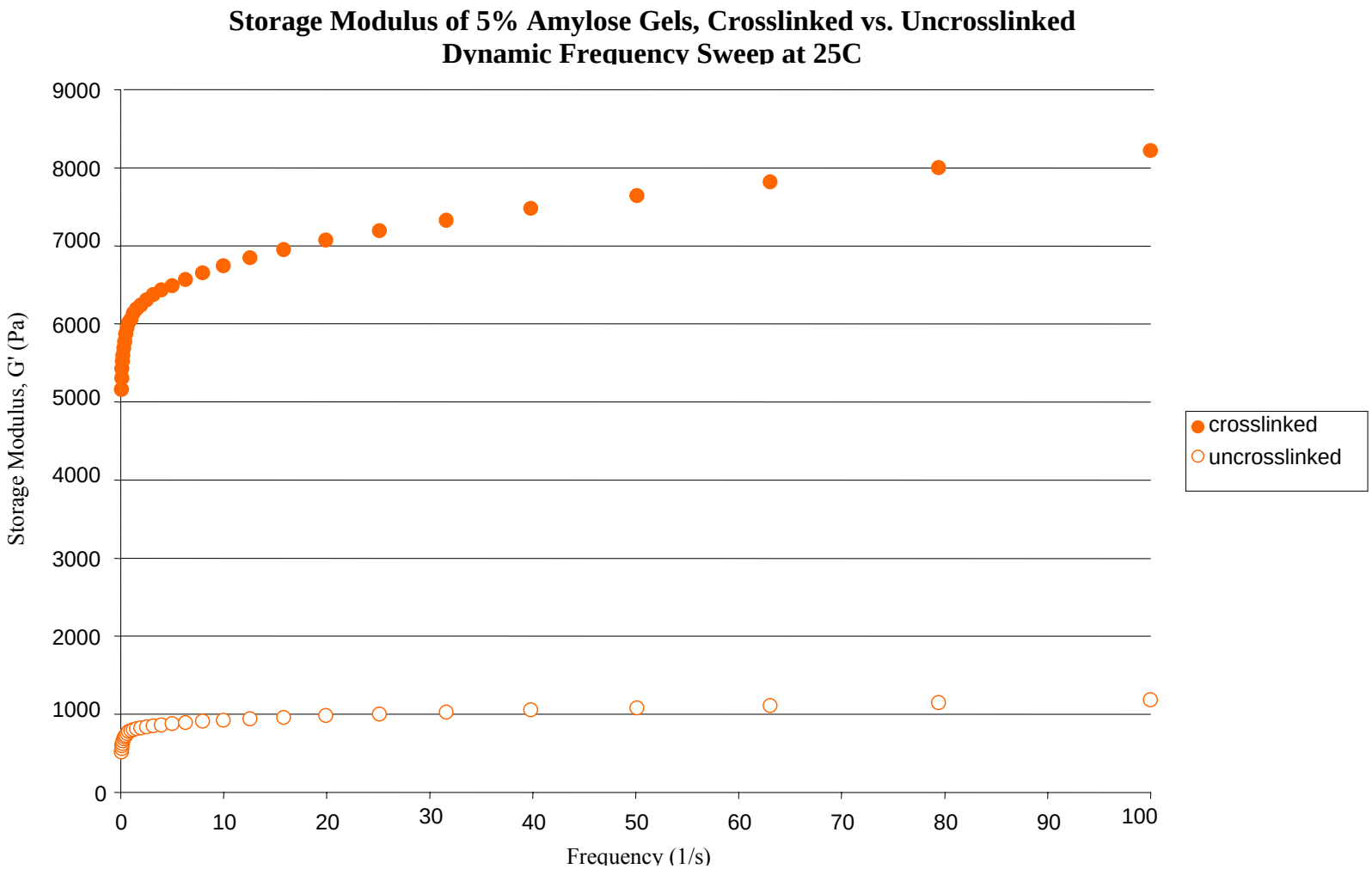


Figure 21. Storage Modulus of Amylose Gels

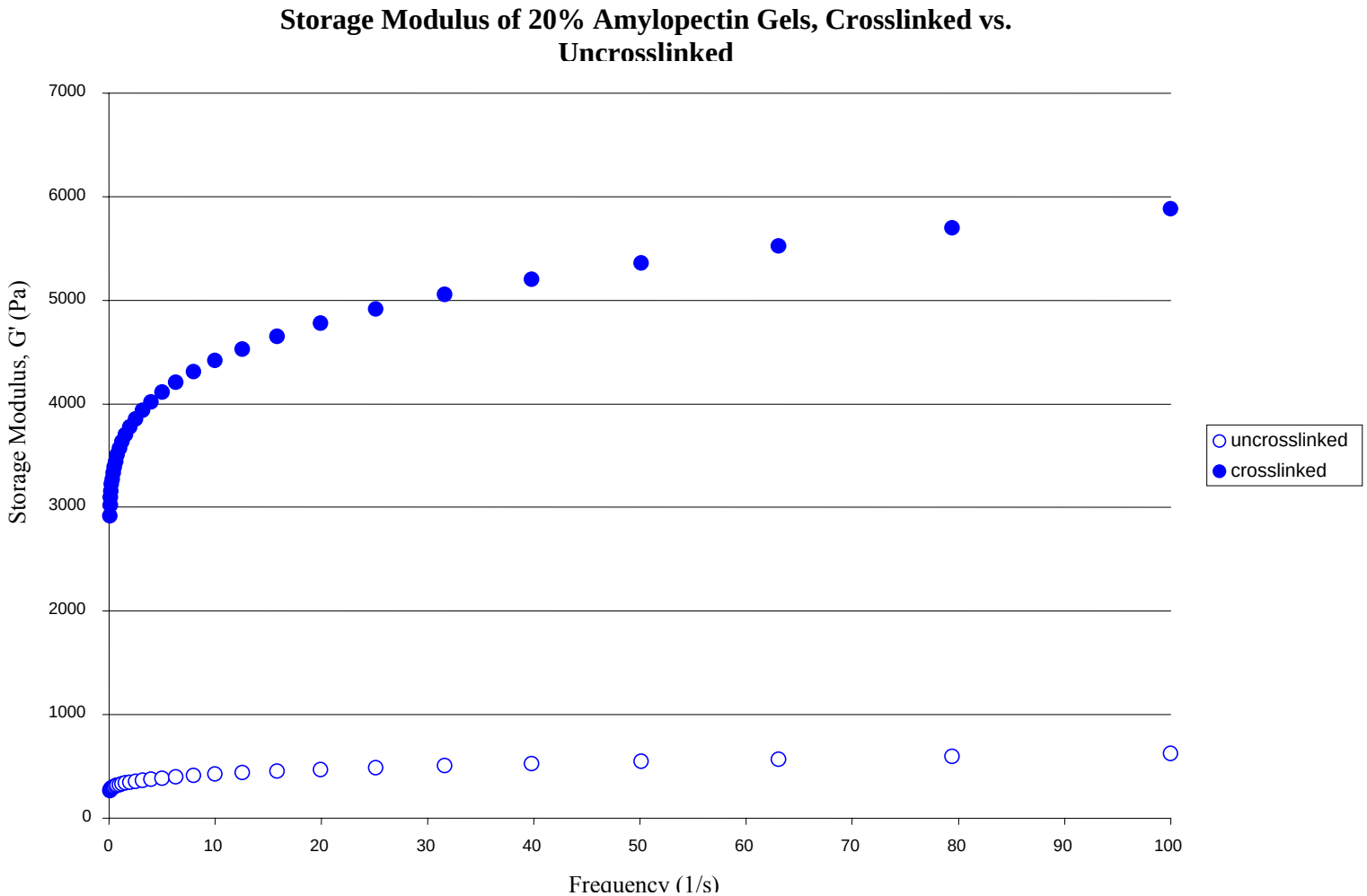


Figure 22. Storage Modulus of Amylopectin Gels.

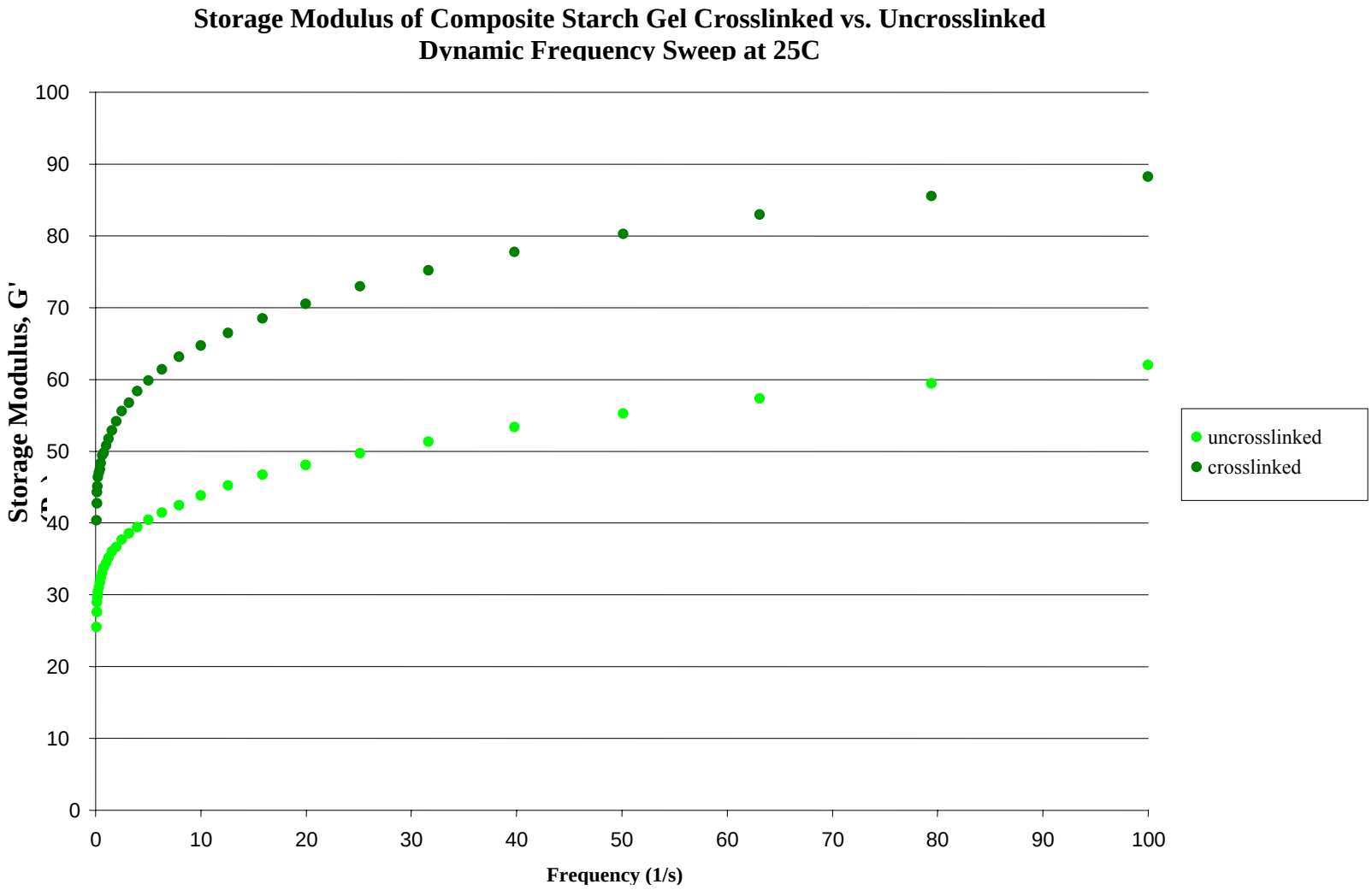


Figure 23. Storage Modulus of Composite Gels.

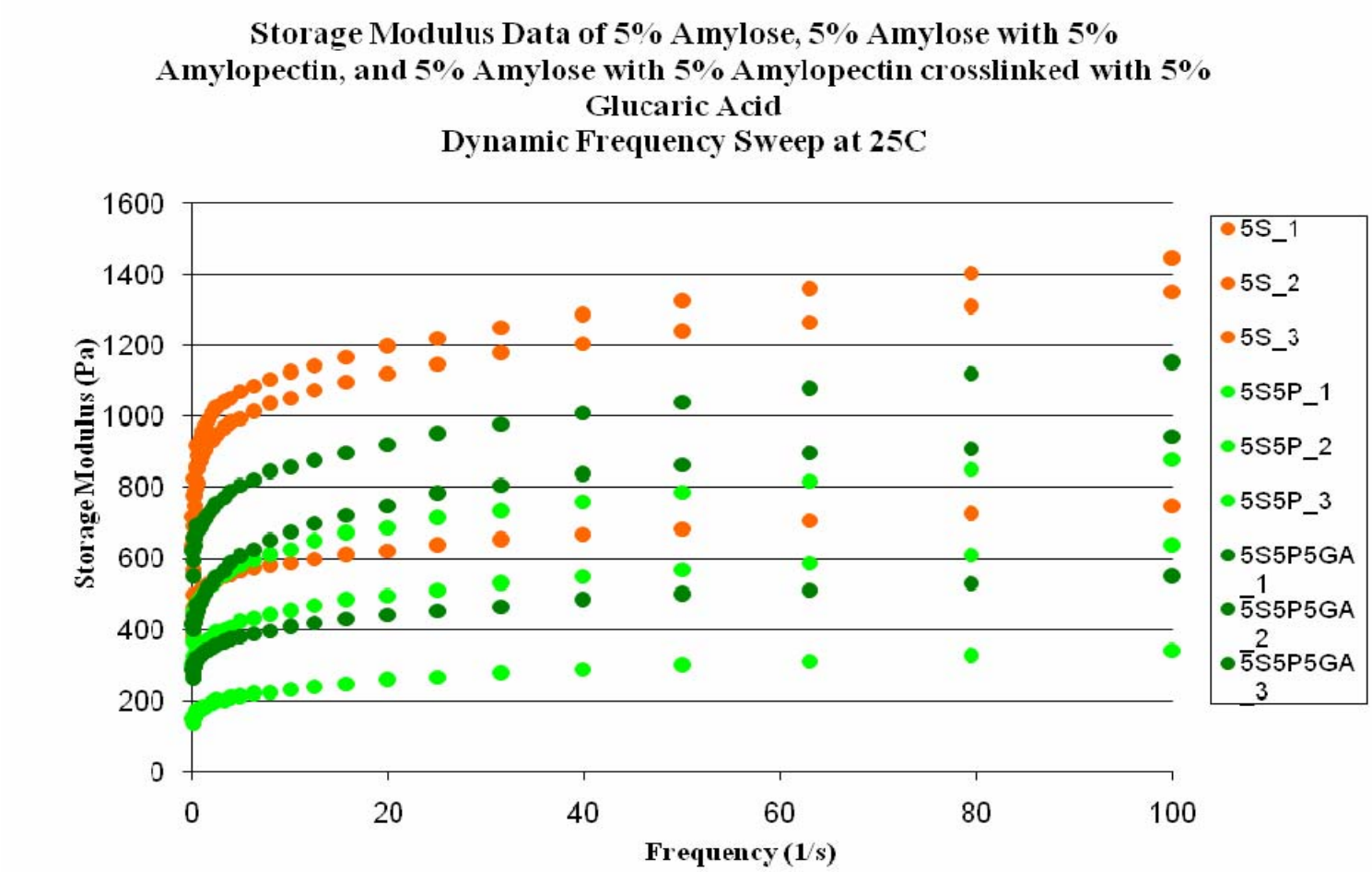


Figure 24. Storage Modulus of Composite Gels.

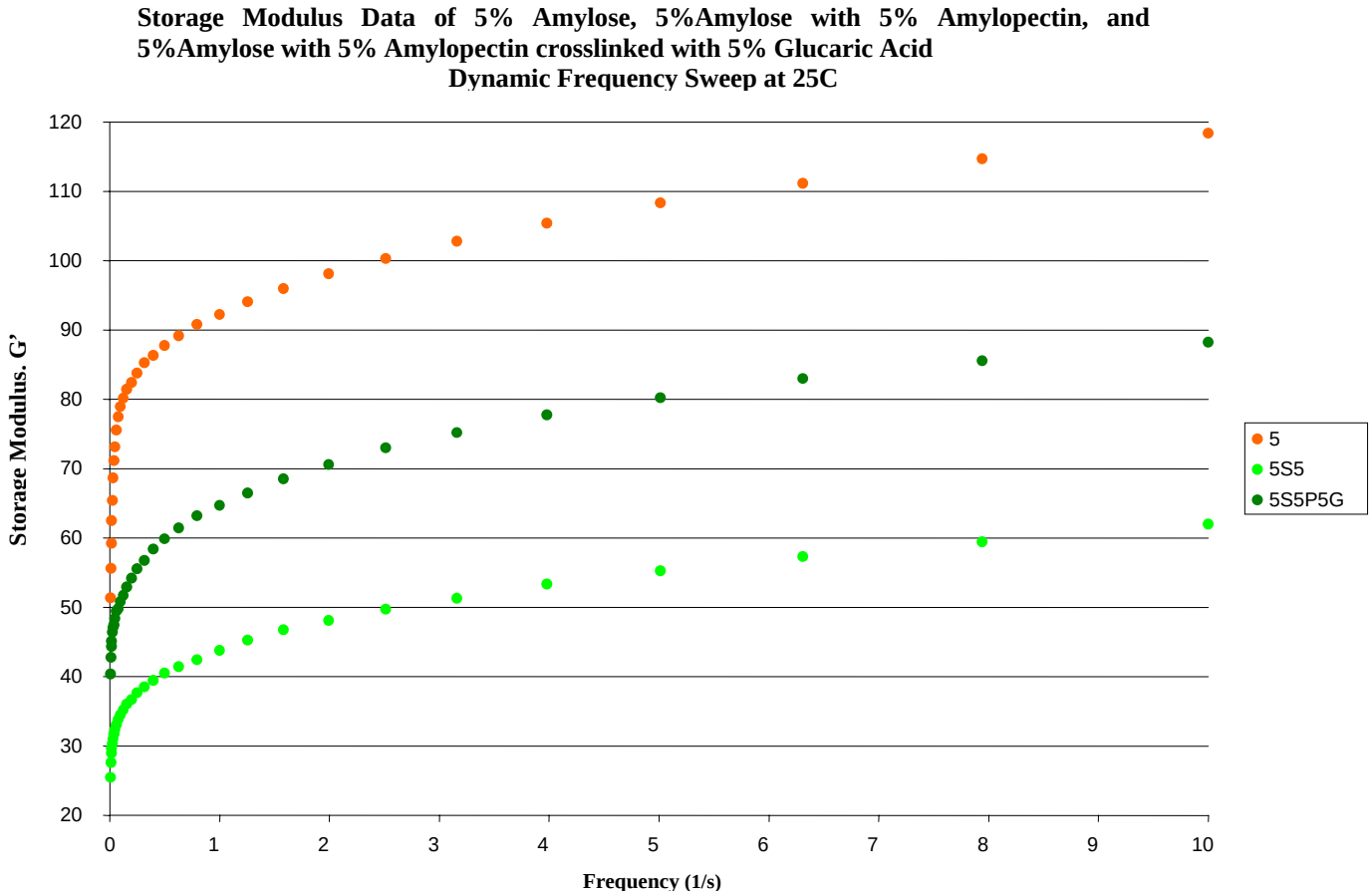


Figure 25. Storage Modulus of Composite Gels.

The storage modulus data relates to Equations 40 for bifunctional crosslinking agents, the number average molecular weight between crosslinks, M_c, may be determined by the relation

$$M_c = \frac{M_r}{2CR} \tag{40}$$

which is the ratio of molecular repeat units to twice the crosslink ratio. This crosslink ratio is determined by the number of reacted crosslinking monomers which would be directly related to the concentration of crosslinker in the gel. The increase in crosslinking of the system would decrease the average molecular weight between crosslinks of the network, and from Equation 22

$$G = \frac{\rho RT}{\overline{M_c}} \frac{\overline{r_0^2}}{\overline{r_f^2}} \left(1 - \frac{2\overline{M_c}}{\overline{M_n}}\right) \tag{22}$$

the Elastic Modulus, G, is inversely related to the molecular weight between crosslinks. So, as the number of crosslinks increases, the average molecular weight between crosslinks decreases, and the modulus of the system increases. From these relationships, the increase in modulus between crosslinked and uncrosslinked gels is due to the chemical bonds formed by the crosslinker of the system.

Degradation

A schematic of the crosslinked starch gel is illustrated in Figure 26. In the illustration the groups of helical chains represent the physically entangled starch chains the for aggregates. The black lines are the exposed starch chains connected the aggregates and forming the network. The blue lines are the glucaric acid crosslinks. The crosslinks are illustrated both within the aggregates and between aggregates. It is assumed that the glucaric acid reacts with the –OH groups on the outside of the starch helices but it has not yet been determined if the di-acid reacts before or after the formation of the starch aggregates during synthesis. If the chemical crosslinking occurs after the formation of the aggregates, then the bonds formed by the di-acid will reinforce the network formed by the aggregates. If the chemical crosslinking occurs before the formation of aggregates, then the aggregates themselves are chemically crosslinked. From the data collected in this work, it is believed that the chemical crosslinking occurs simultaneously and that there are both crosslinked aggregates and di-acid crosslinks reinforcing the network.

The degradation of the gel appears to occur in two stages. Because the gels are made of aggregates, physically entangled starch molecular chains that serve as junction points of the network, the first stage of degradation is the hydrolytic degradation of the exposed chains holding the aggregated starch chains together. The aggregates dissolve in the second stage of degradation because it takes more time for the water to diffuse into the physically entangled mass. So, after the first stage of degradation, the network is broken and the aggregates are free to behave more like particles and precipitate out of solution.

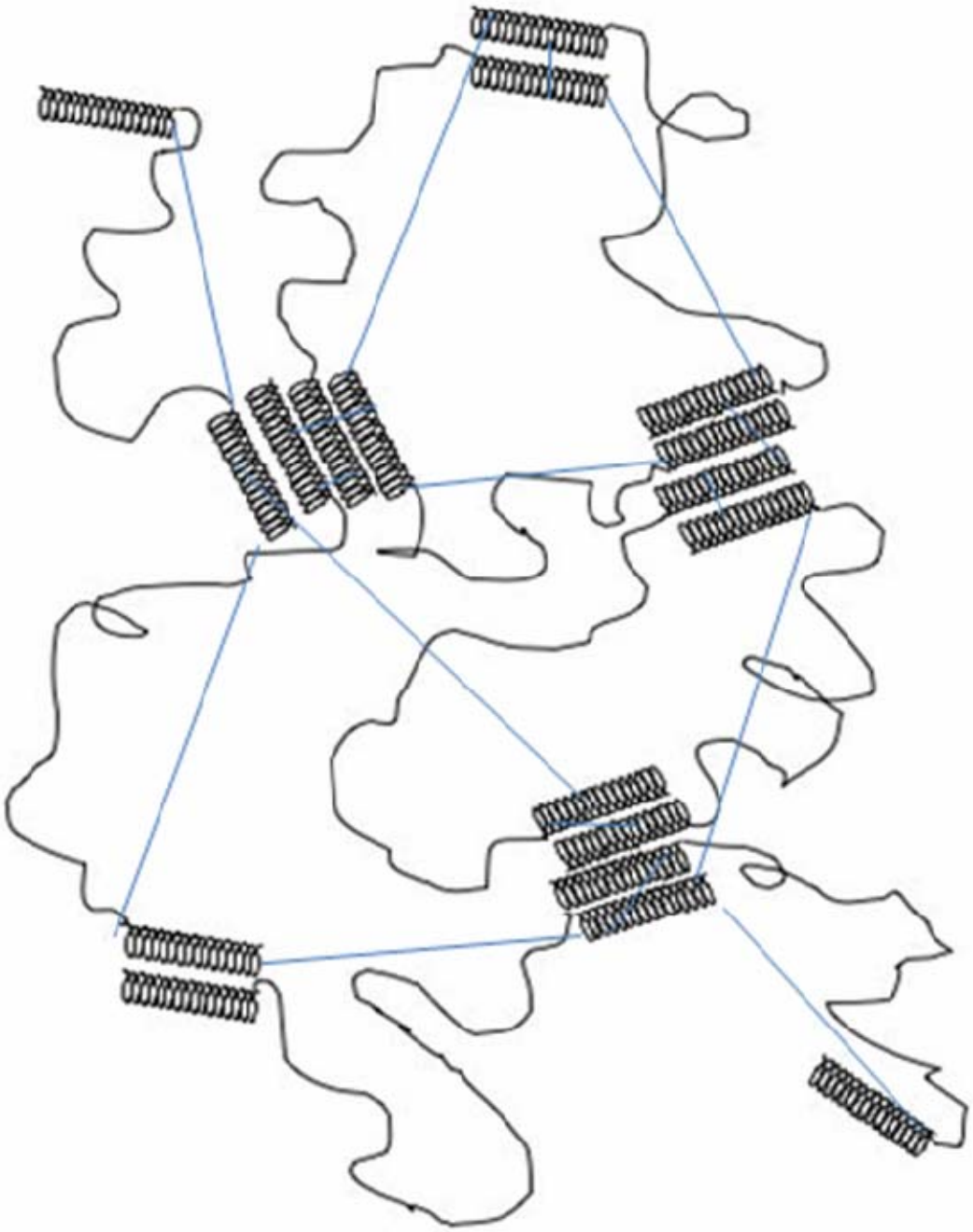


Figure 26. Schematic of Crosslinked Starch Hydrogel.

Visual observation reveals the un-degraded gel is a single mass with a 3-dimensional structure whereas the degraded gel appears to be particles that have precipitated out of solution and clung to the sides as well as settled to the bottom of the beaker. Figure 27 is the un-degraded gel and Figure 28 is after the gel has been submerged in cell culture media for 5 days.

Figure 29 is of degraded gels beginning with the uncrosslinked starch, 0.25% crosslinked starch, 0.5% crosslinked starch, and 5% crosslinked starch after 5 days in fetal bovine serum. From these pictures, not only is there obvious sedimentation of the aggregates in most of the gels, but there is also noticeable differences in the color of the solutions. A pH indicator is added to the culture media and turns from red to yellow when the pH changes from neutral to acidic.

All of the solutions in Figure 29 were red in color when the gel was added to the media. The 5% solution changed to yellow within 24 hours. The 0.5% solution changed steadily over the 5 day span. The control and 0.25% samples did not change. From these images, it is not only evident that the gel is degrading, but in the 5% solution there is unreacted glucaric acid monomer present within the matrix hence the immediate color change. The evidence of the unreacted monomer is the early change in pH that only occurs in the 5% sample. The steady change in color in the 0.5% solution and the precipitation of aggregates implies that there is a steady release of acid into solution first by diffusion of the unreacted monomer and secondly by the hydrolytically degradation of the chemical bonds formed by the glucaric acid crosslinker.

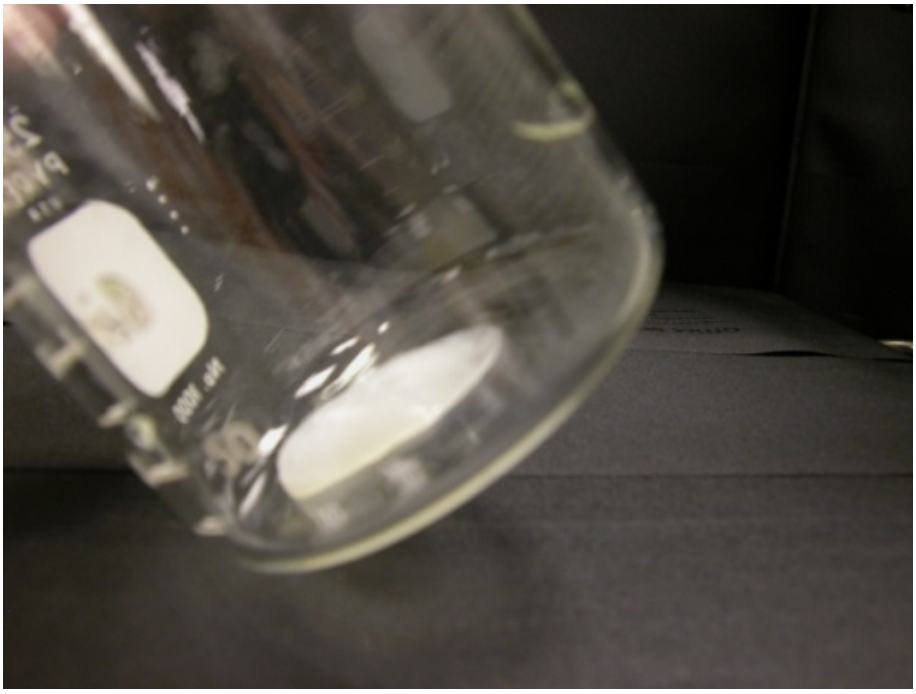


Figure 27. Undegraded Amygel.

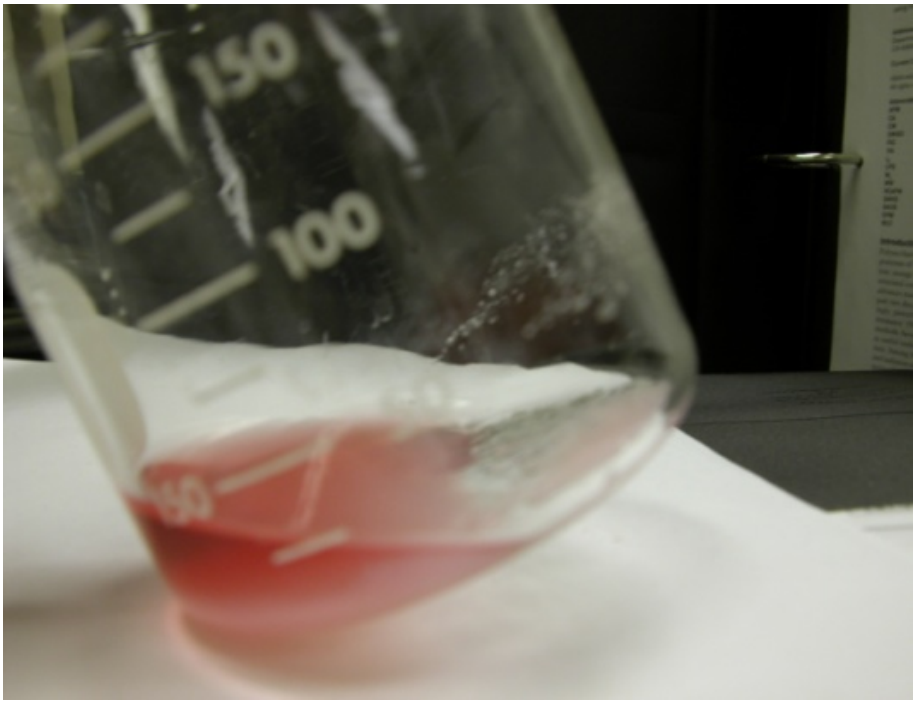


Figure 28. Degraded Amygel.

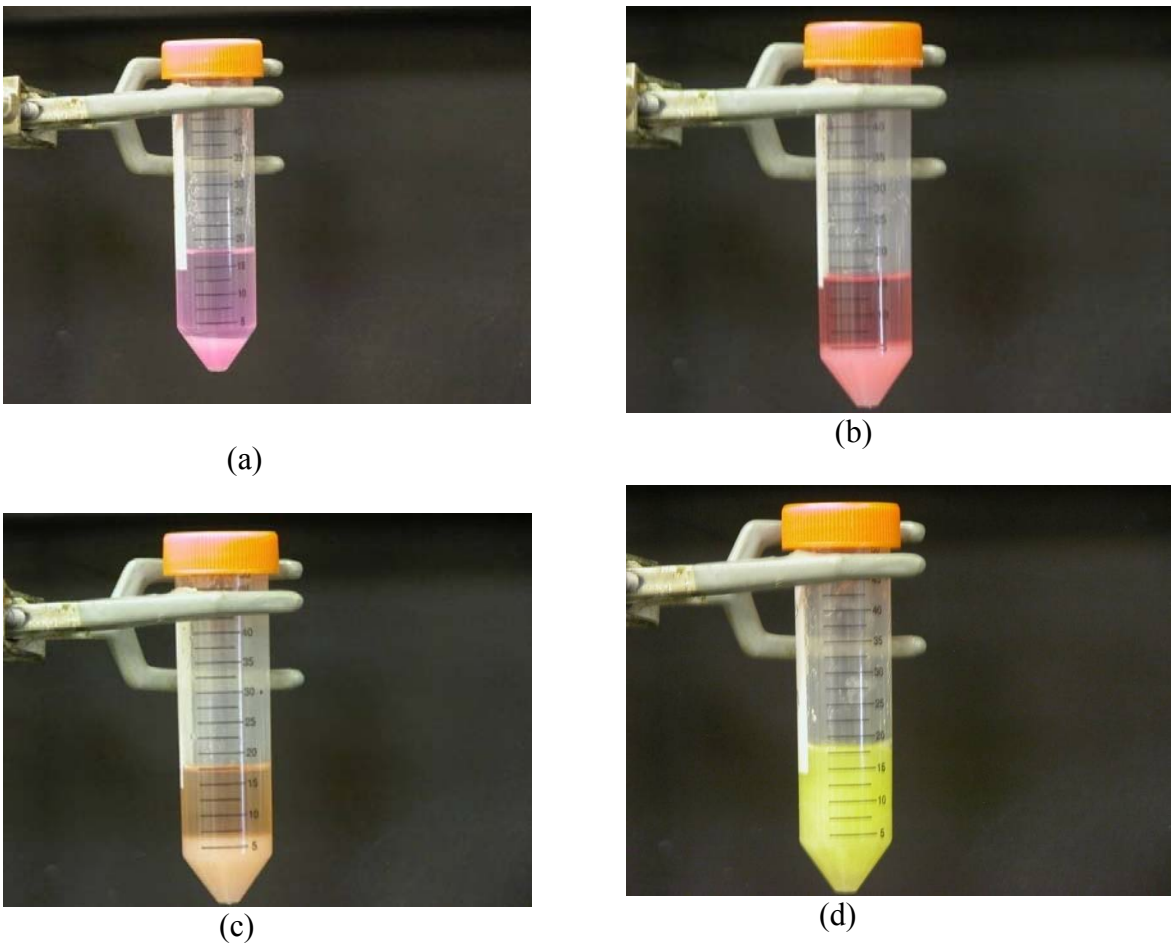


Figure 29. Amygel after Degradation in Cell Culture Media- (a) starch, (b) 0.25% crosslinked starch, (c) 0.5% crosslinked starch, and (d) 5% crosslinked starch.

From these two pieces of evidence, the changes in pH and the precipitation of particles, the gel appears to be a combination device of both the Chemically Controlled Release System and the Diffusion Controlled Release System described earlier in this text. The two step theory of the degradation mechanism also lends itself to the idea manipulating the amounts of unreacted and reacted crosslink monomer to achieve constant or zero order release kinetics also described earlier.

It should also be noted that the 5% crosslinked gel remains a gel with some sedimentation of particles on the bottom of the test tube after the 5 day experimental period implying that the increased crosslink concentration has extended the degradation time of the 5% crosslinked gel. This data may be evidence that the chemical bonding of the glucaric acid does play a role in reinforcing the network considering the network of the 5% crosslinked gel remains a network after the other samples have degraded into particles.

The pH of cell culture solutions was measured during the course of the 5 day proliferation study, and changes in pH of solution were present. The shifts in pH of the culture media are so slight, however, that the amount of acid being released from the system could not be quantified. Although only slight changes were detected, the data does illustrate an obvious trend. As illustrated in Figure 30.

This slight shift from neutral is encouraging evidence for both the release of acid and also the idea that the cell proliferation is decreasing, as described in the next section, as an effect of the drug because these changes are so slight that cell death is probably not due to extreme environmental conditions.

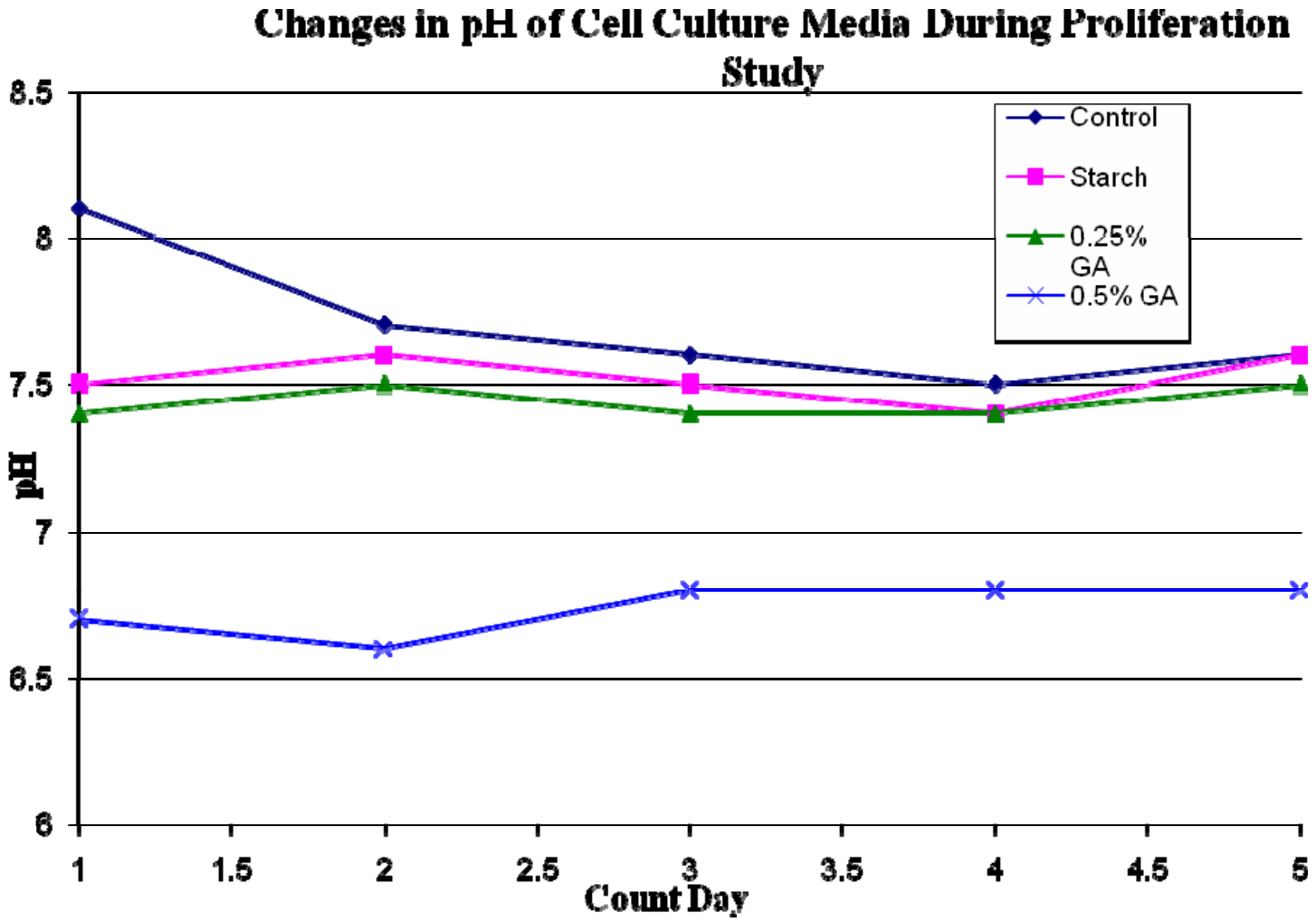


Figure 30. Changes in pH of Cell Culture Media During Proliferation Study

Cell Culture Studies

Although the colony studies were inconclusive because there were too many colonies to count, qualitative visual data was obtained from the fixed cells. There appear to be no apparent differences in the cell morphology of treated versus untreated cells, as illustrated in the sample images in Figure 31 implying that the drug does not cause physical stress to the cells but instead induces some sort of cell cycle response. Additional images of the Colony Study cultures are presented in Appendix B.

The results of the growth assay are represented graphically below. Figure 32 represents the number of cells after 5 days exposure to glucaric acid alone. From the figure, the varying concentrations of d-glucaric acid in the cell culture media affected the proliferation of the U2OS cells by 30-70%. Figure 32 represents the growth curve of the U2OS cells exposed to the crosslinked gel. 250,000 cells were plated and allowed to rest for 24 hours before the addition of the crosslinked gel to the culture medium. The samples were then counted daily to estimate the number of cells in each plate.

The growth curves of the control plates and the uncrosslinked starch plates appear similar. The plot illustrates increased suppression of cell proliferation with increased glucaric acid crosslink concentration. The 1% glucaric acid composition impedes cell proliferation of osteosarcomas by 70% after 4 days exposure. Figures 33 and 34 are bar graphs comparing cell proliferation studies at the same time period. Both cultures were treated with the same amount of glucaric acid. In one culture, the glucaric acid was dissolved in the media and added to the culture. In the second, the same amount of glucaric acid was reacted with starch, and the crosslinked starch gel was added to the culture media. The decrease in cell proliferation of both treated samples appear similar.

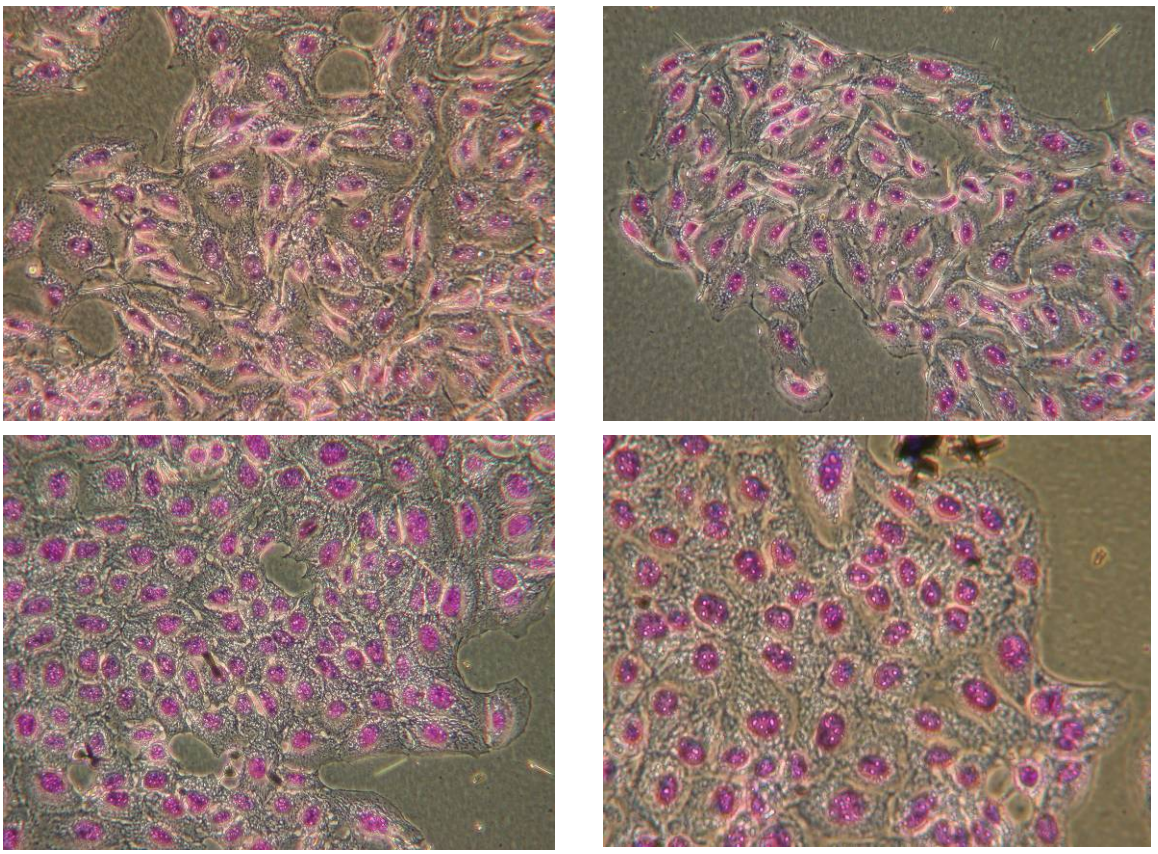


Figure 31. Cell Cultures Exposed to Varying Concentrations of Glucaric Acid.

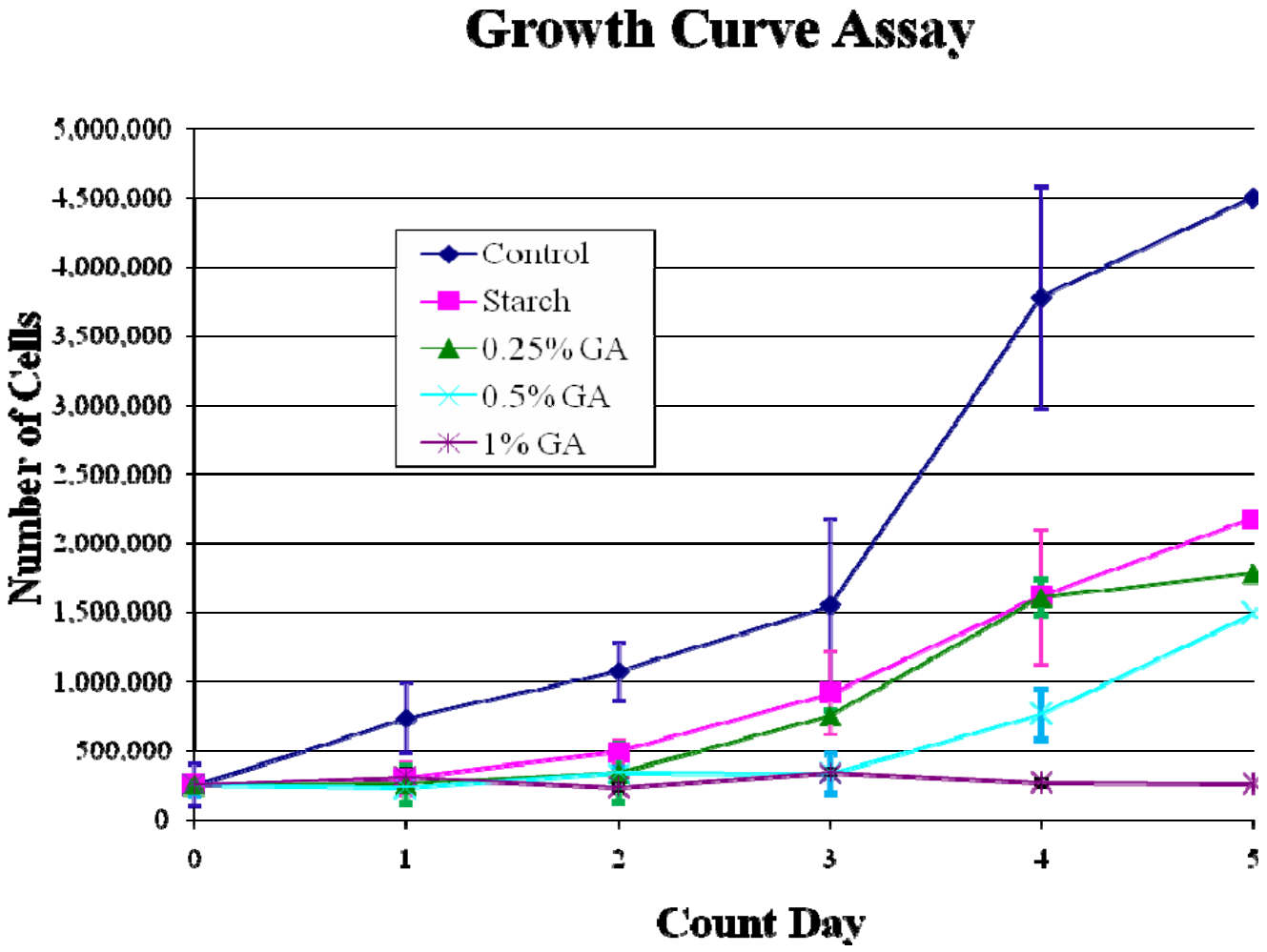


Figure 32. Cell Proliferation During 5 Days Exposure to Amygel

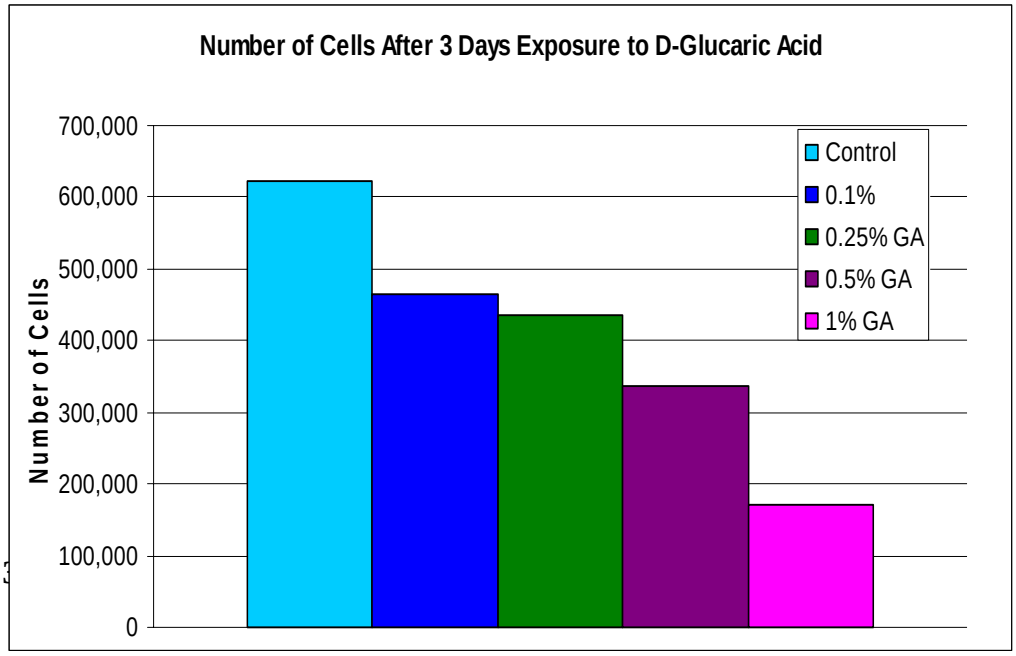


Figure 3

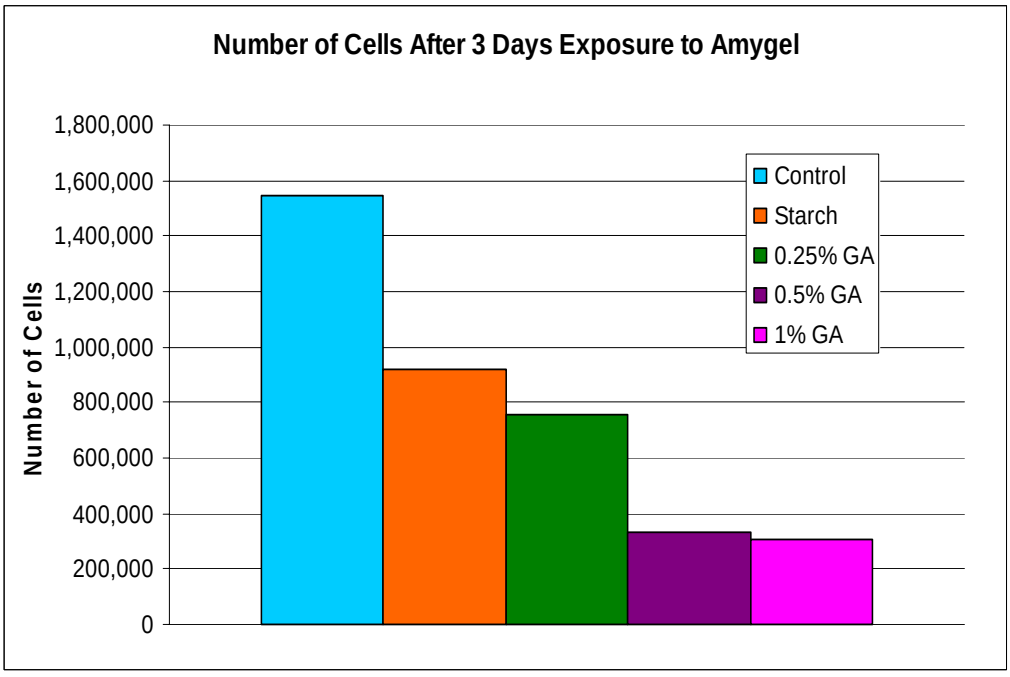


Figure 34. Number of Cells after 3 Days Exposure to Amygel

V. Conclusions and Future Work

Conclusions

- A polymeric network of amylose and amylopectin, the main components of starch, was formed and chemically crosslinked with d-glucaric acid.
- By crosslinking the amylose and amylopectin gel with d-glucaric acid the elastic modulus of the system increases.
- By crosslinking the amylose and amylopectin gel with d-glucaric acid the degradation time of the gel is extended.
- The amylose and amylopectin gel as well as the amylose and amylopectin gel crosslinked with d-glucaric acid, Amygel, are biodegradable.
- Upon degradation, the chemical crosslinker, d-glucaric acid, present in Amygel is released from the system and decreases cell proliferation of human osteosarcoma cells.
- Before degradation, unreacted d-glucaric acid is stored in the matrix of the hydrogel. This drug is released upon diffusion out of the Amygel system and decreases the cell proliferation of human osteosarcoma cells.
- Applications of Amygel include treating carcinogenic tumors by injecting Amygel in the proximity of the tumor and allowing the d-glucaric acid to decrease the size of the tumor or filling a wound where a tumor has been removed to prevent the metastasis of malignant cells that were not removed with the tumor.

Future Work

It is documented that the molecular machinery for producing glucaric acid is present in healthy cells and absent in cancerous ones. Future work should include experiments with healthy cells to test the toxicity of excess glucaric acid in healthy tissues. The foreseen application of the gel is as an injectable anti-tumor treatment and future work should include whole tumor cultures to measure the effects against a functioning tumor. The next step after whole tumor cultures would be animal studies including mouse models. The gel should be analyzed with NMR to define the exact compounds of the system before and after degradation. Solution Neutron Scattering could also explore the physical and chemical structures of the network. It is documented that starch is able to form complexes with other compounds, and neutron scattering could determine if the unreacted monomer is incorporated inside the helix of the starch polymers as other complexes in the literature. Neutron scattering could also likely determine if the reacted glucaric acid crosslinker is bonded to the –OH groups on the outside of the starch helices.

Preparation methods incorporating microwave have been promising but as yet are unsuccessful as methods of producing the gels. The microwaved gels appear more homogeneous but are not as strong as the ones produced with the traditional method. Microwaveable gels would be beneficial especially in a clinical setting where shelf life and storage of prepared gels could be problematic.

Other authors have used the Kiely method to purify the glucaric acid before incorporating the acid into various materials. Future experiments utilizing this method of purification of the acid would increase reactivity of the acid and result in greater extent of the crosslinking reaction of the gels.

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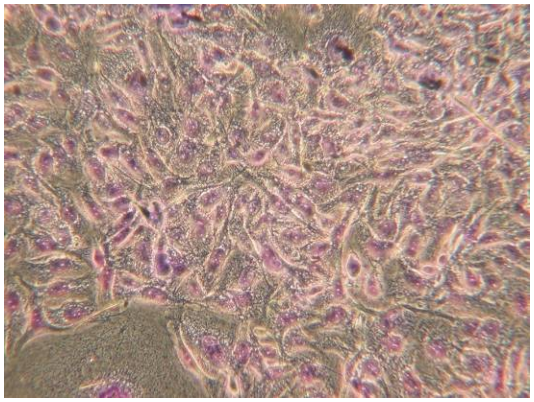
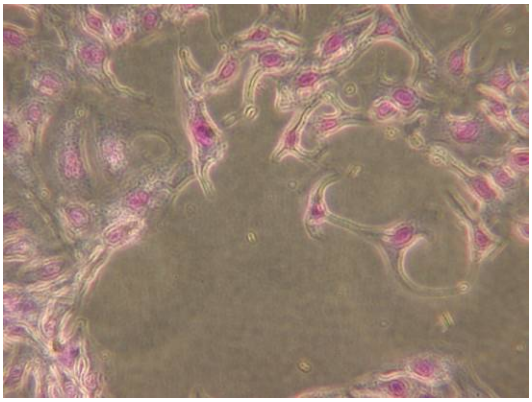
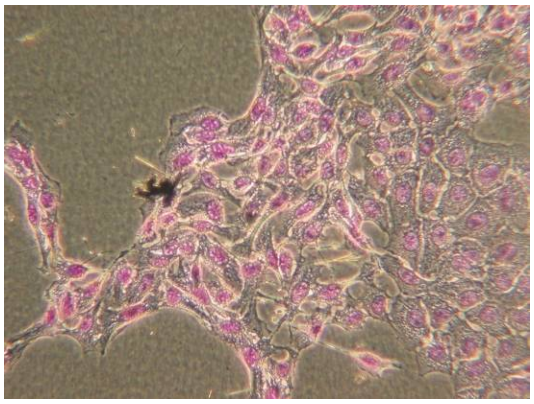
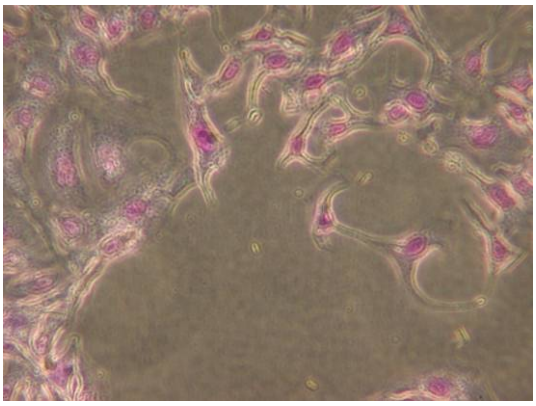
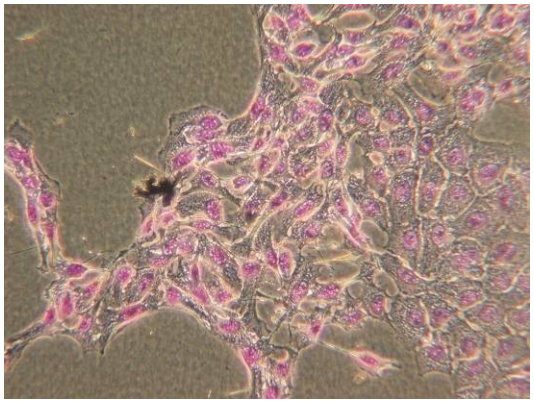
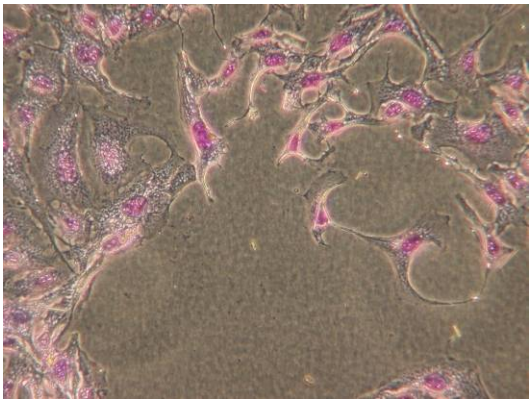
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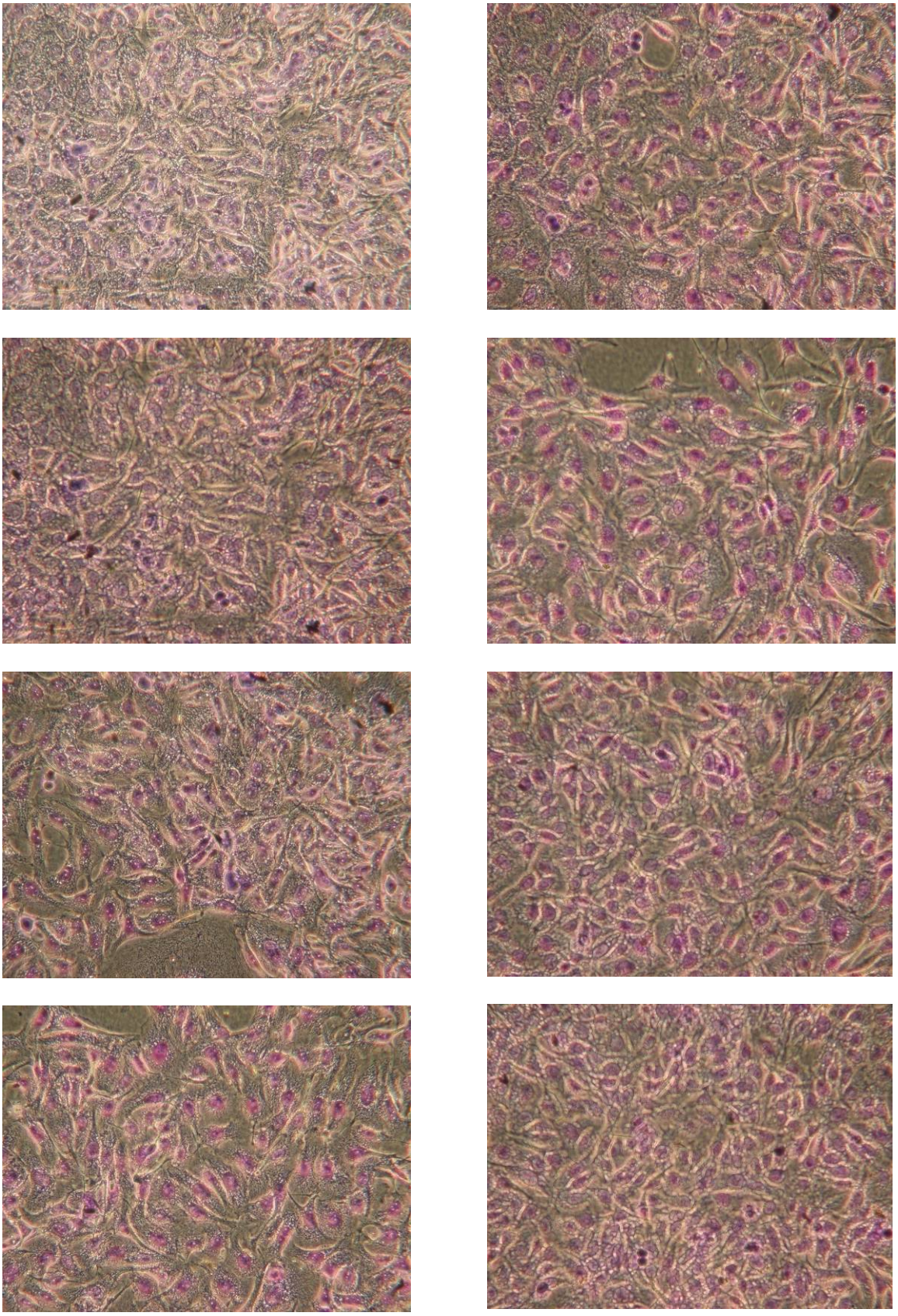
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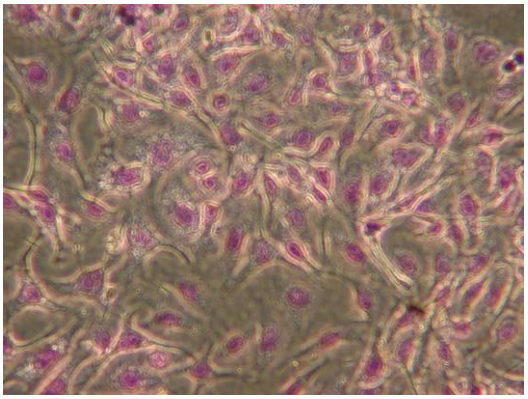
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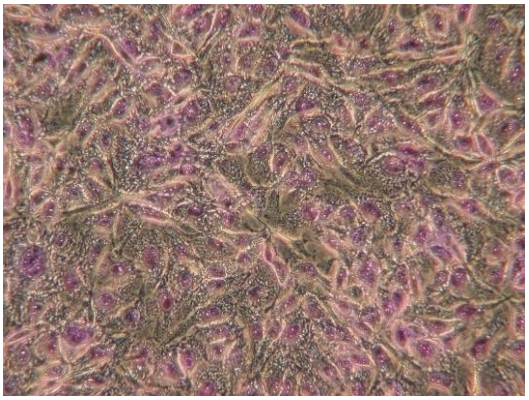
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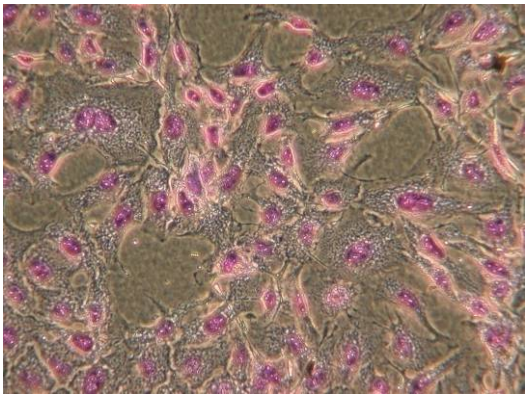
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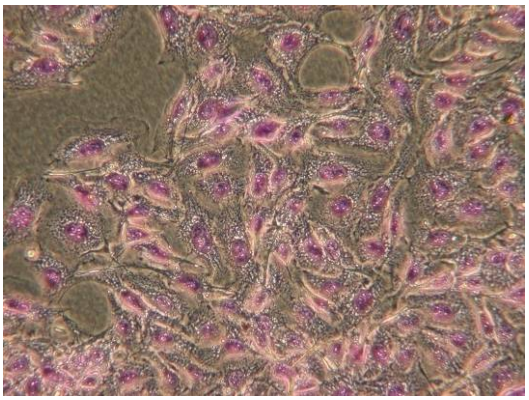







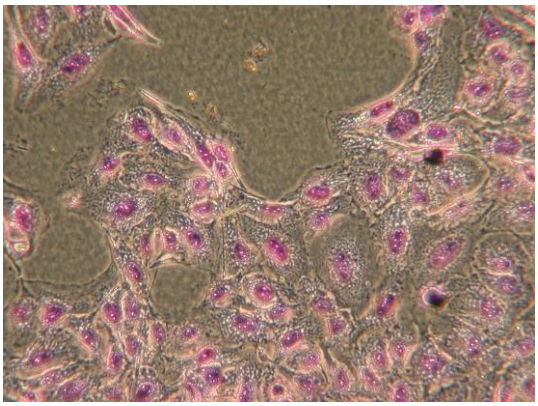


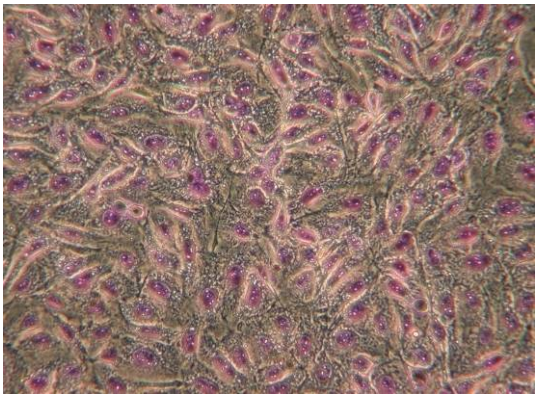






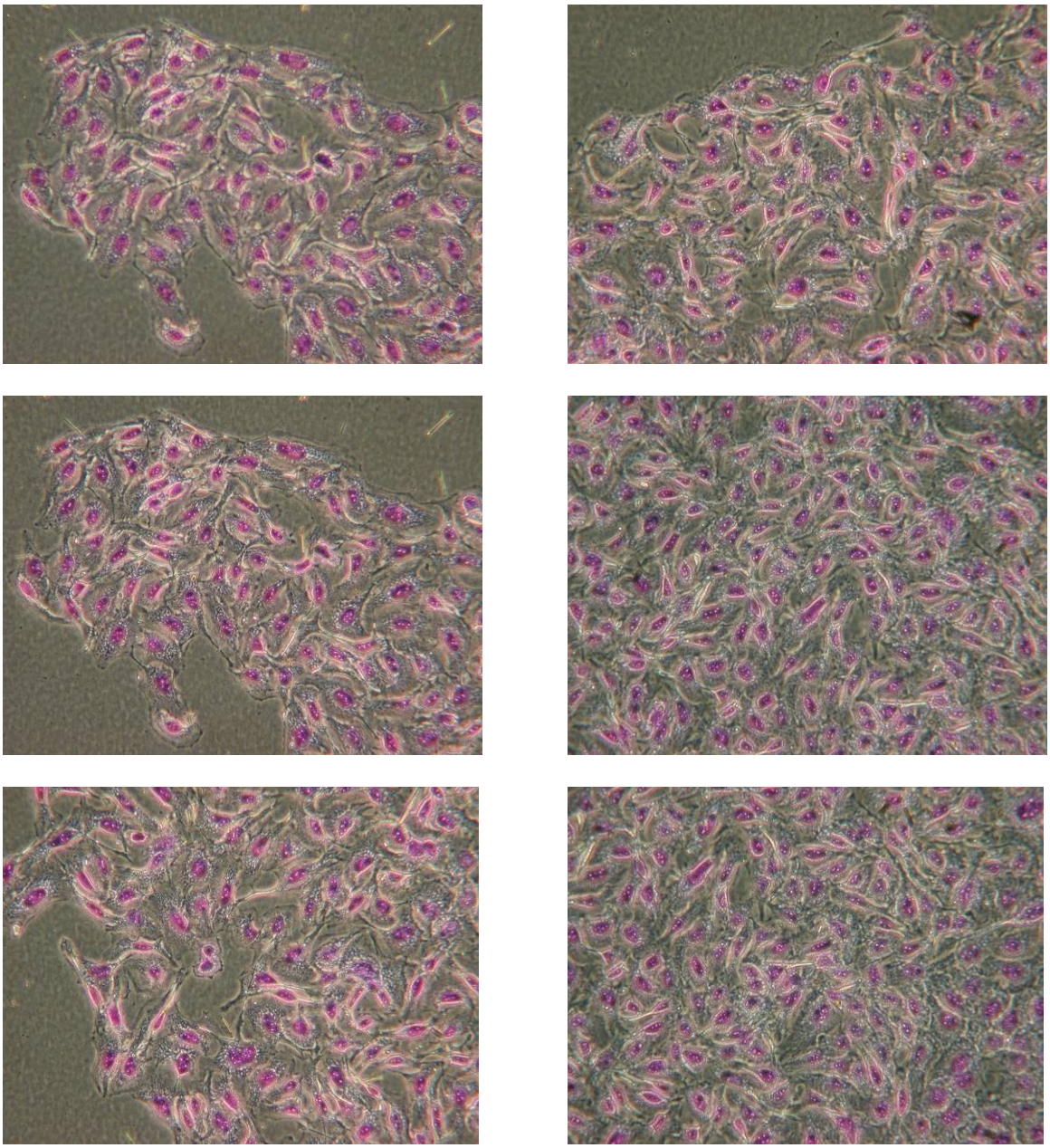


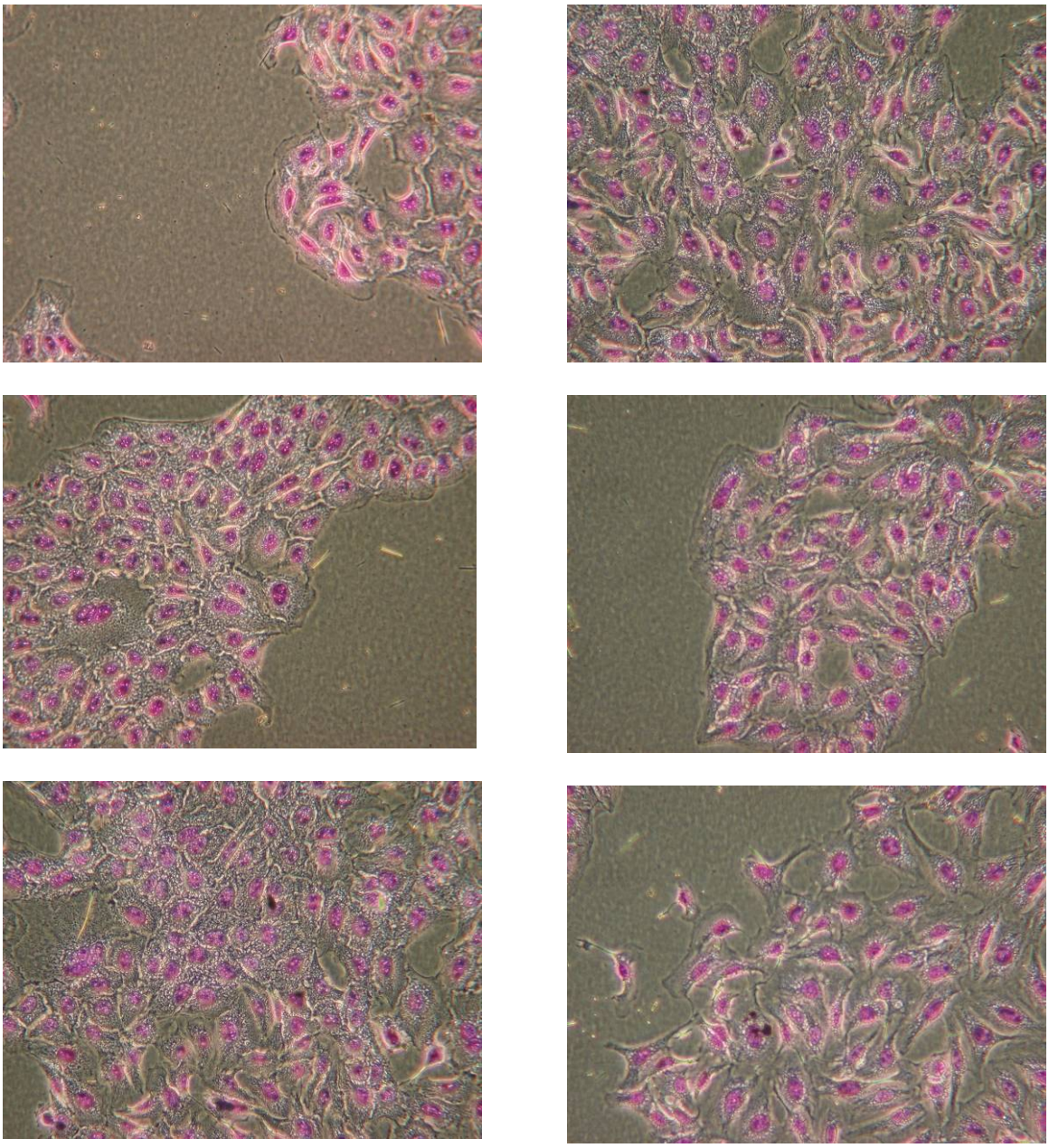


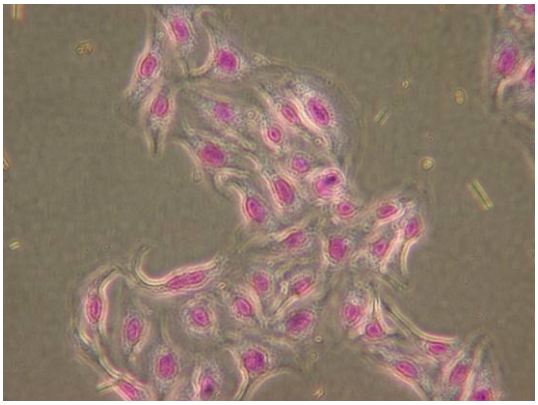
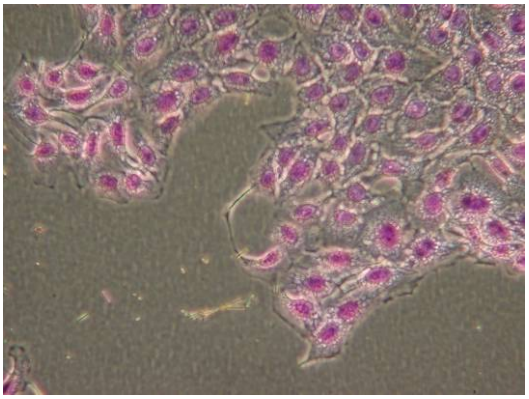
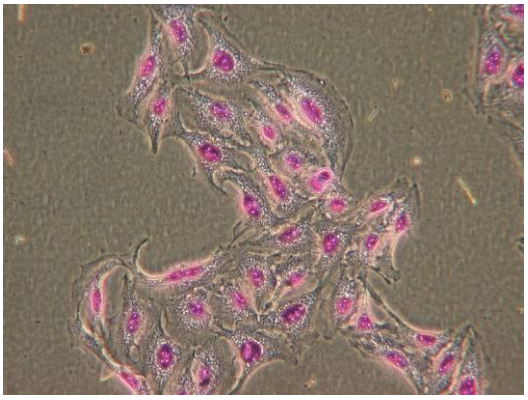
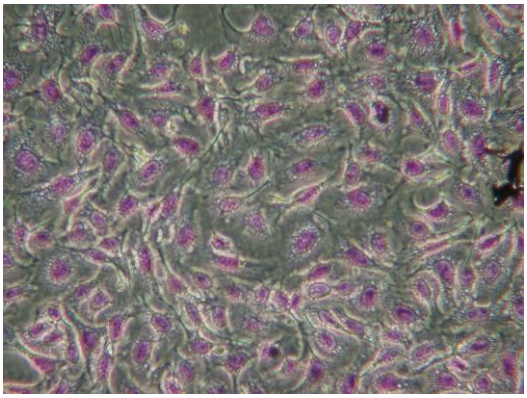
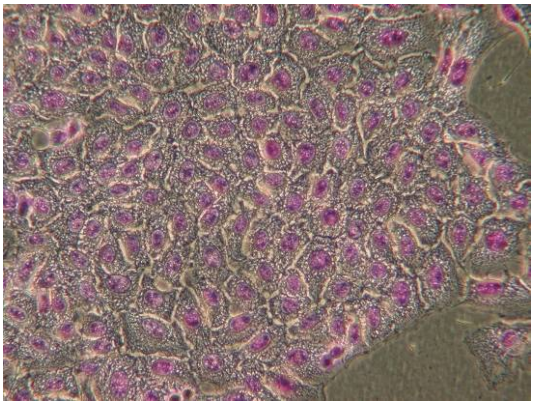
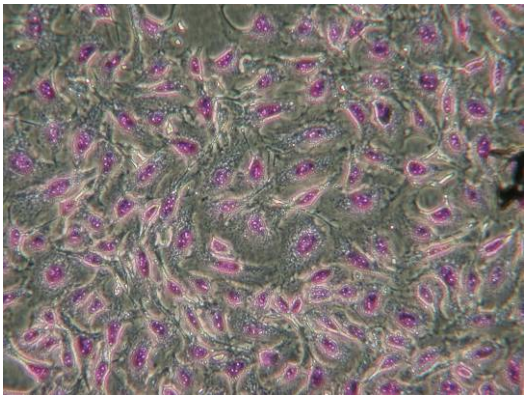


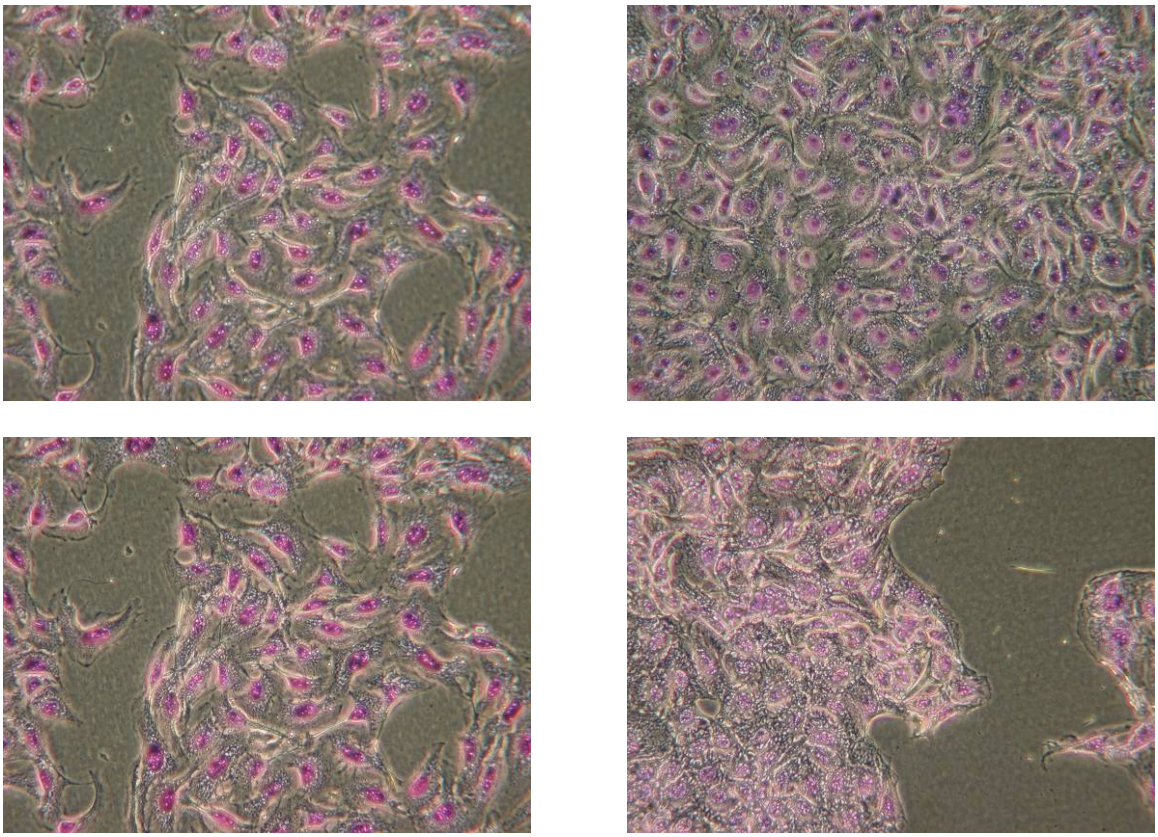
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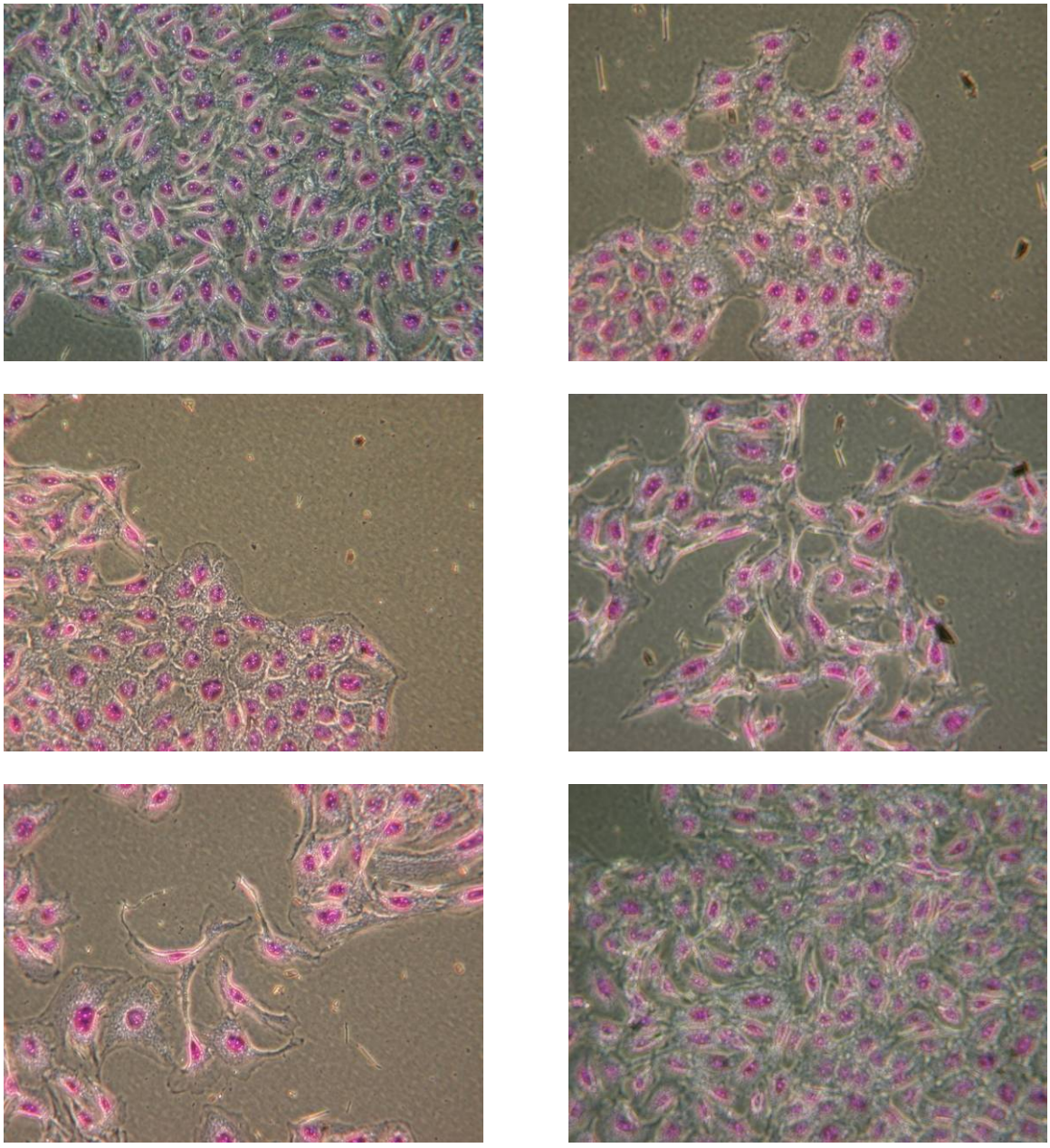


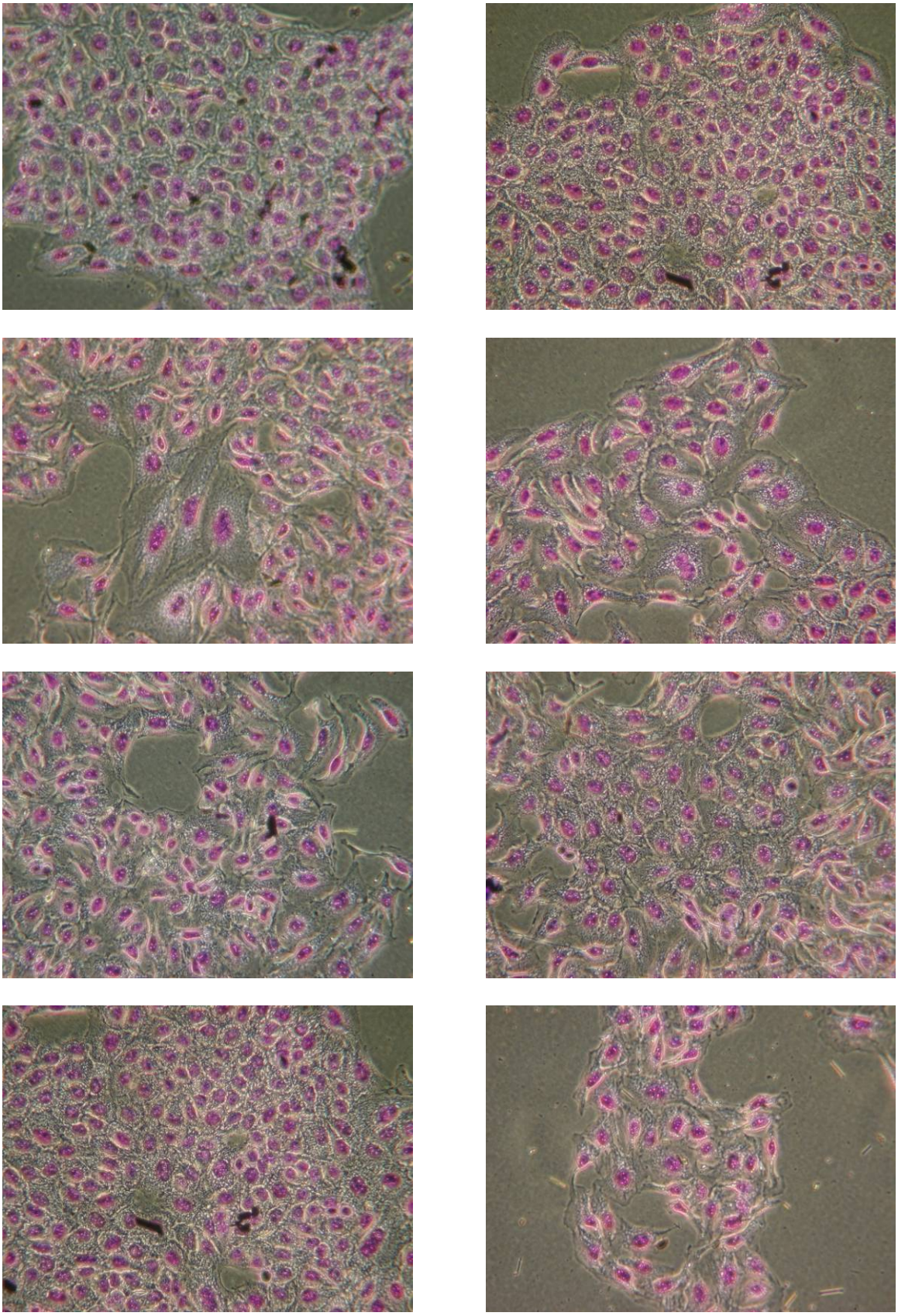







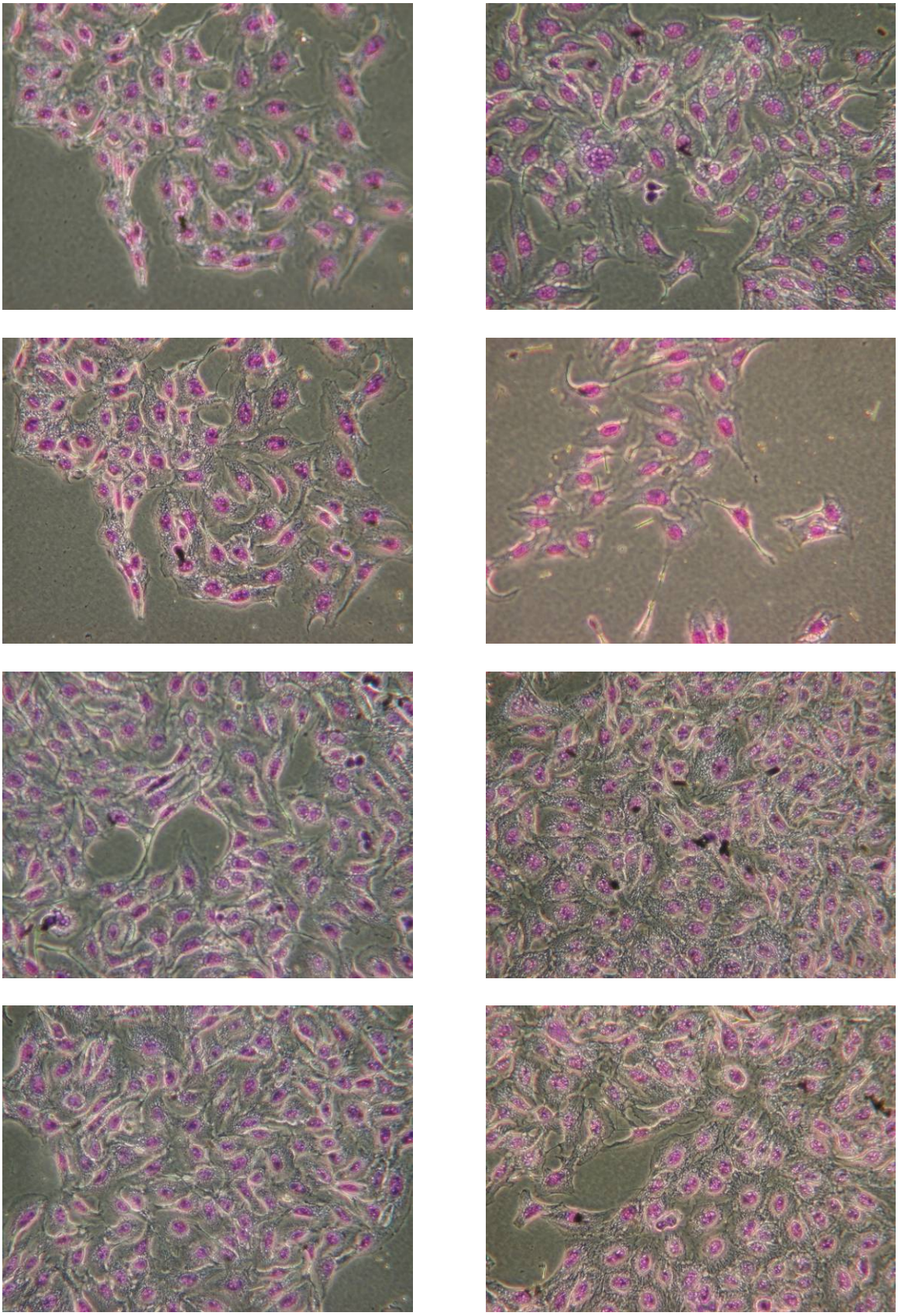


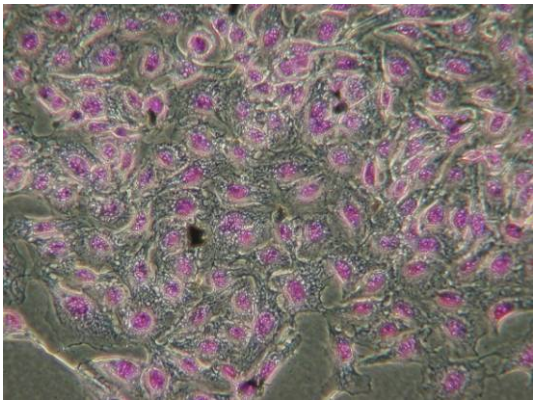
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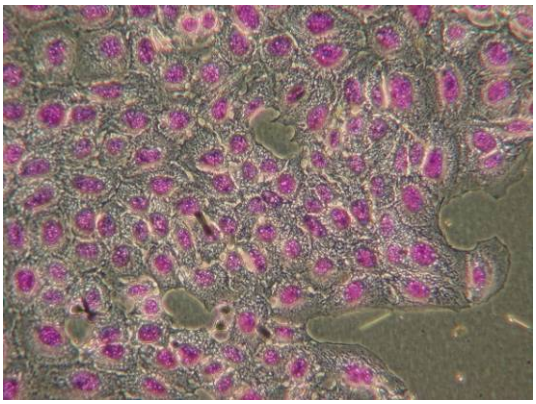
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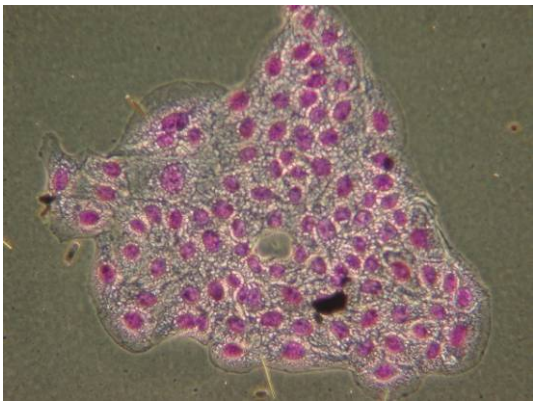


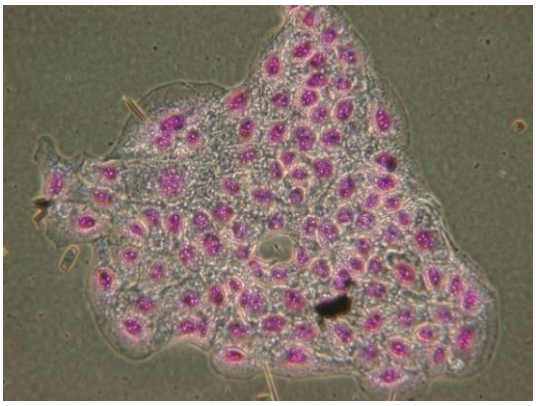






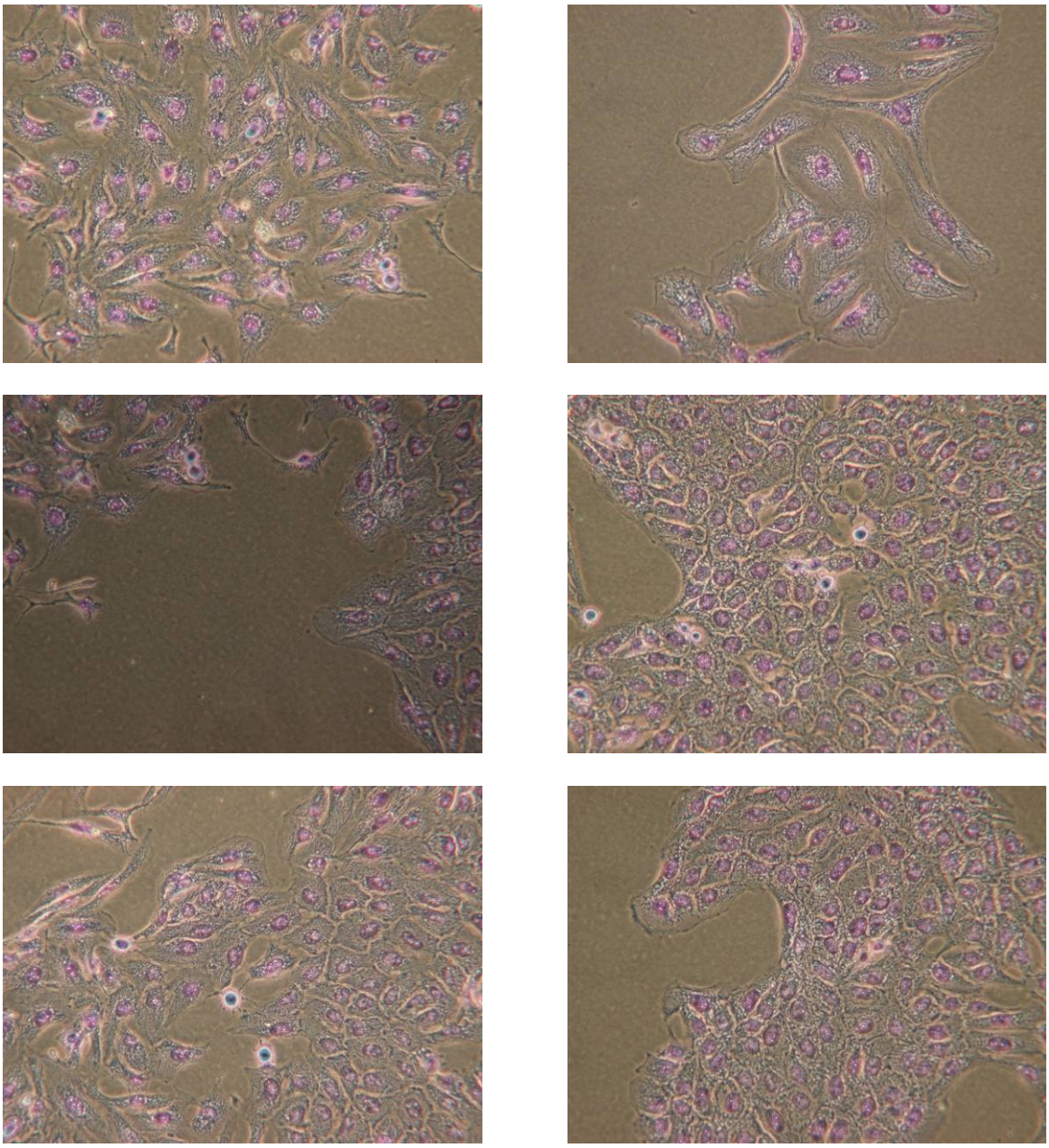


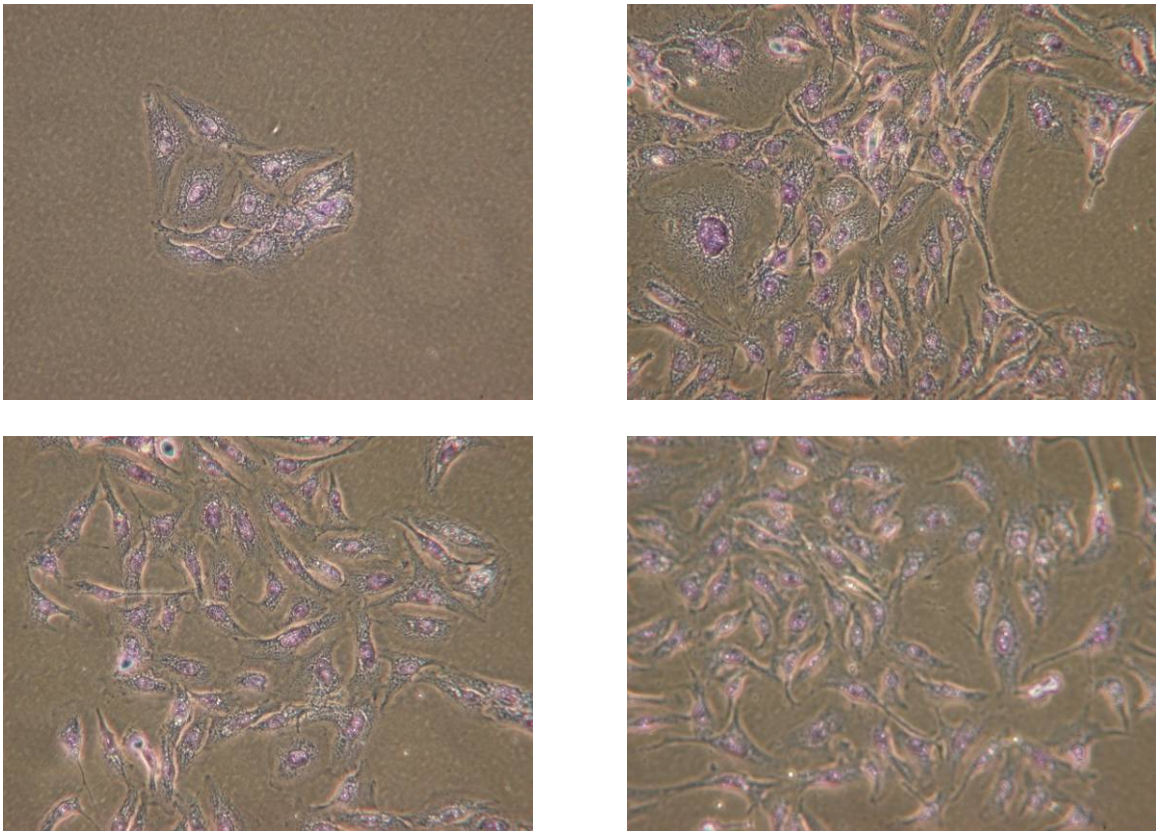




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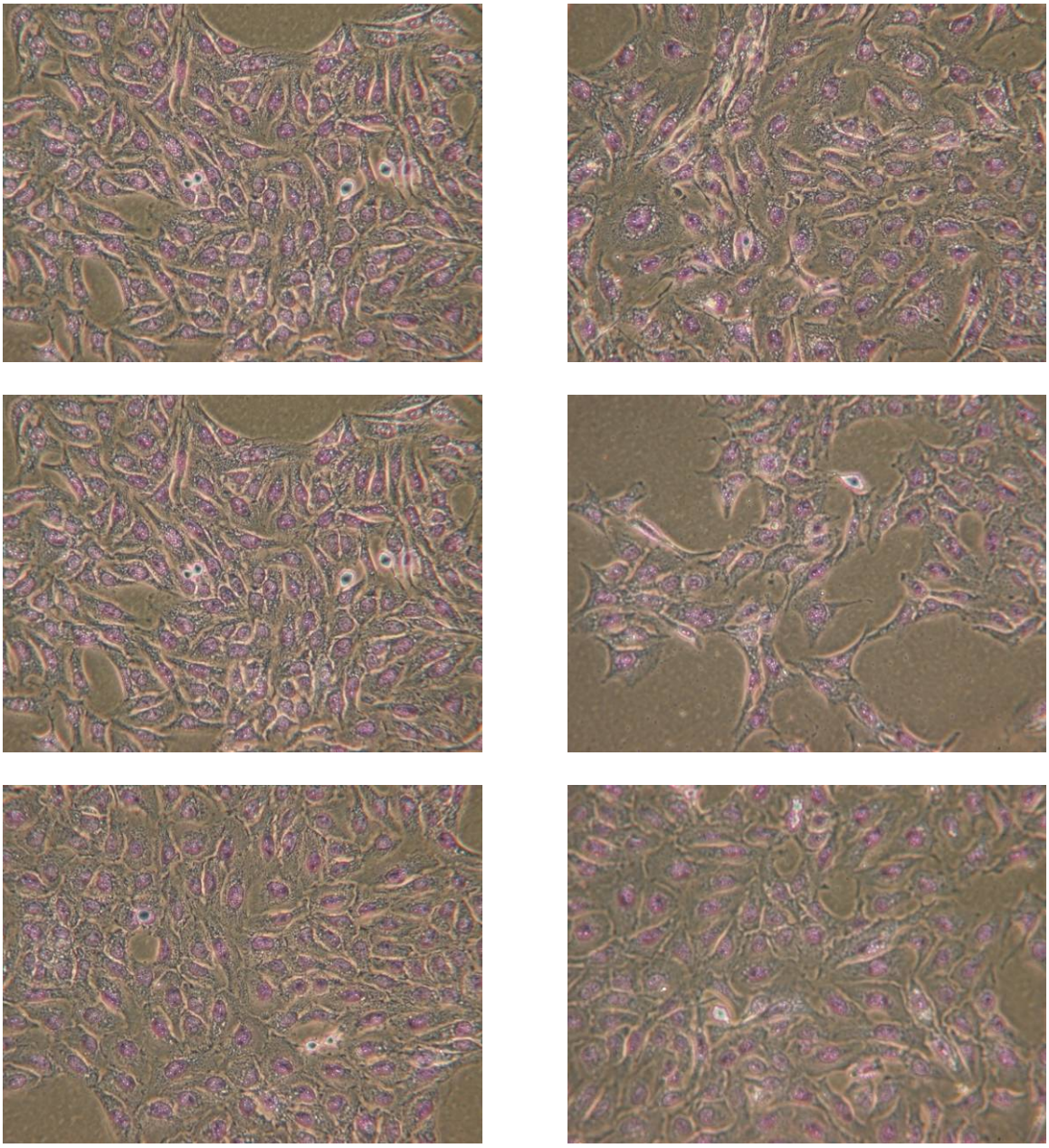
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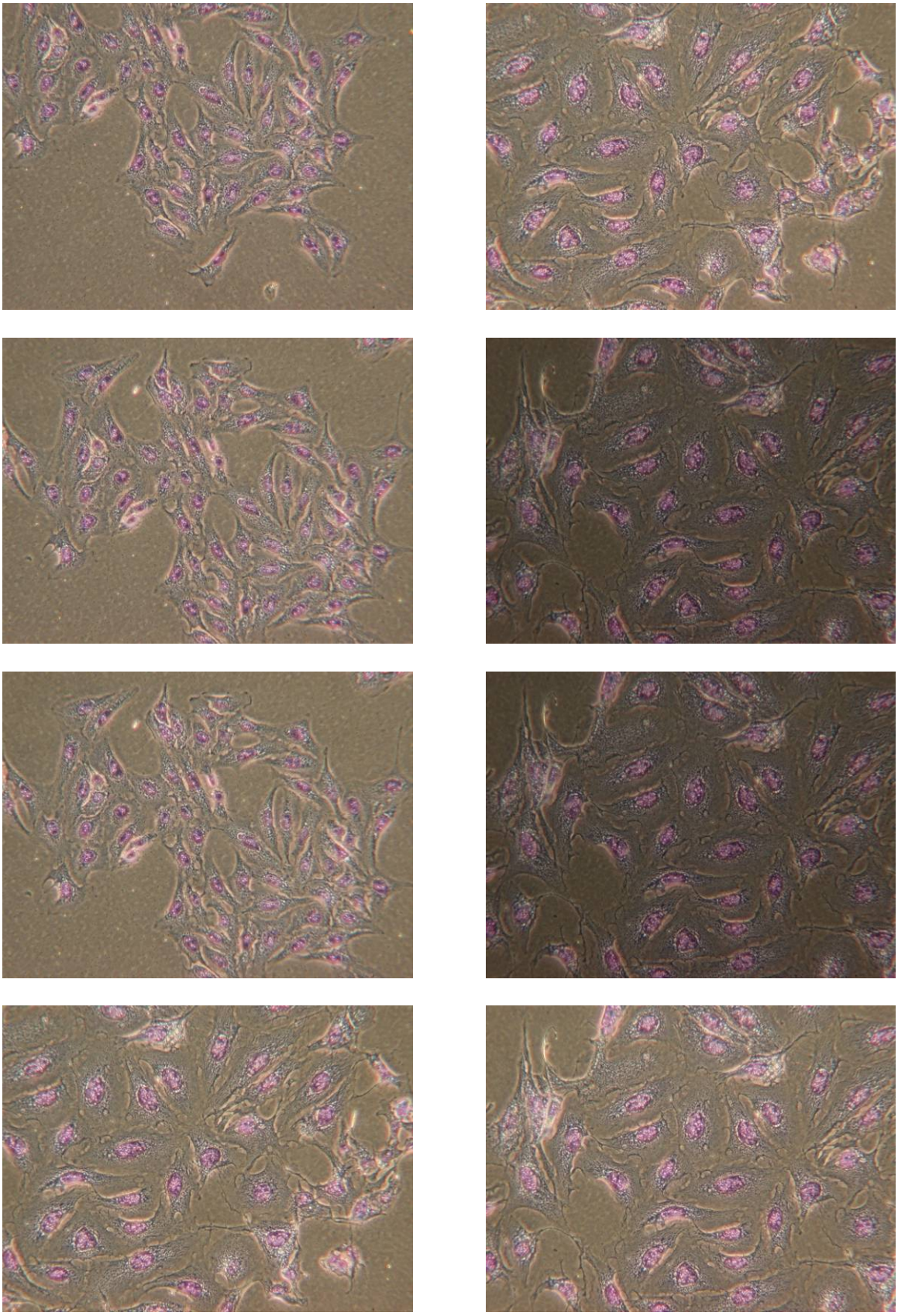


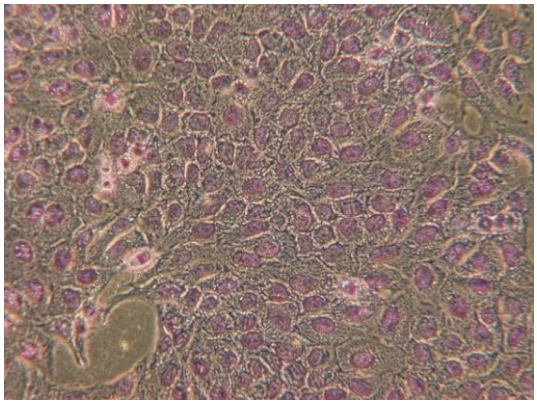


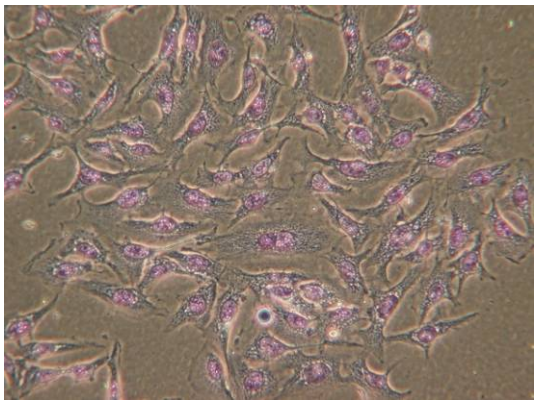
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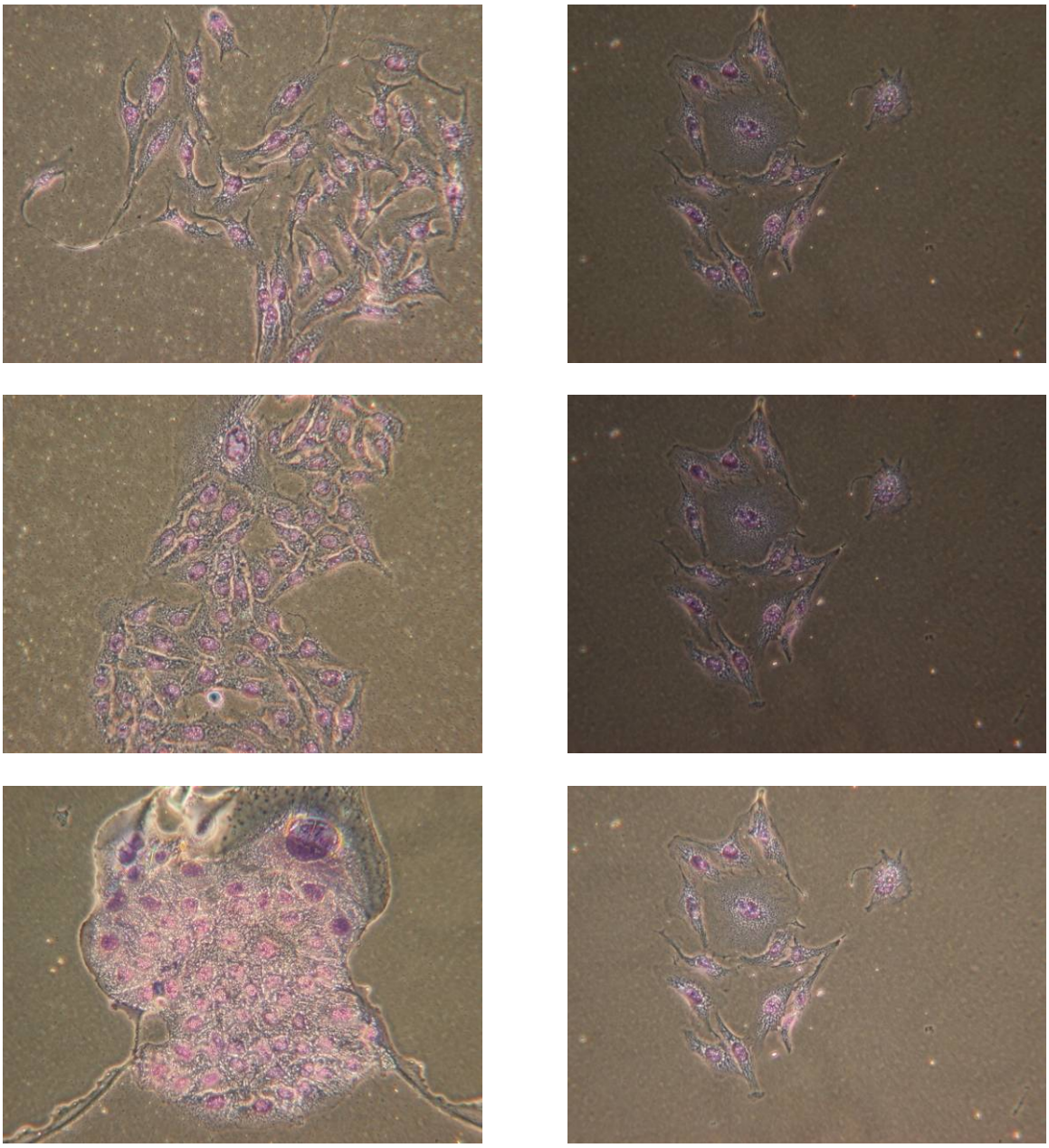


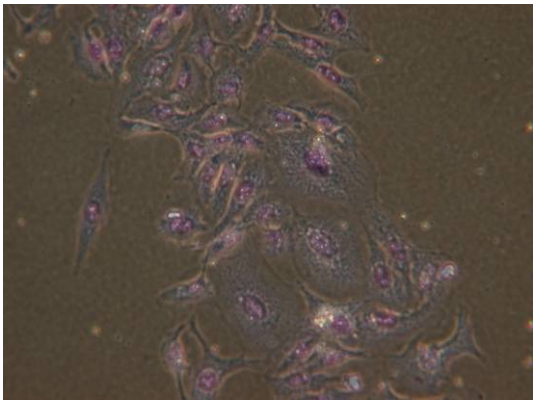


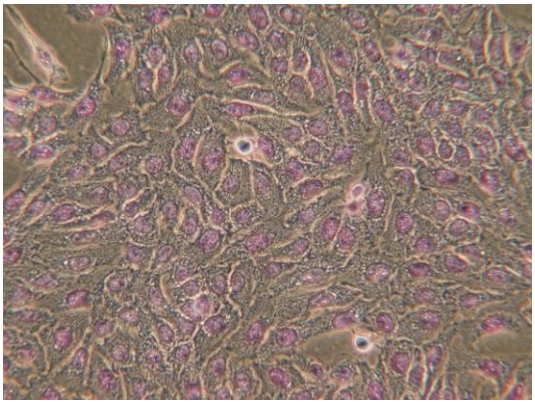


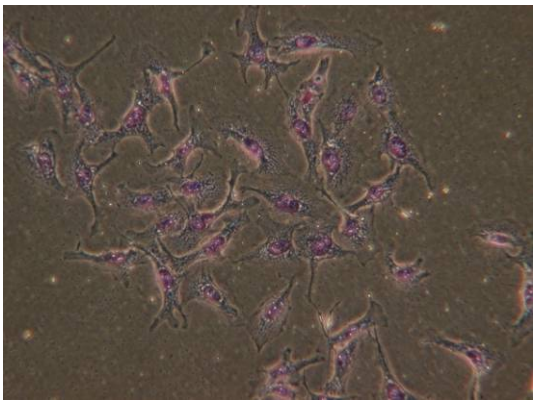
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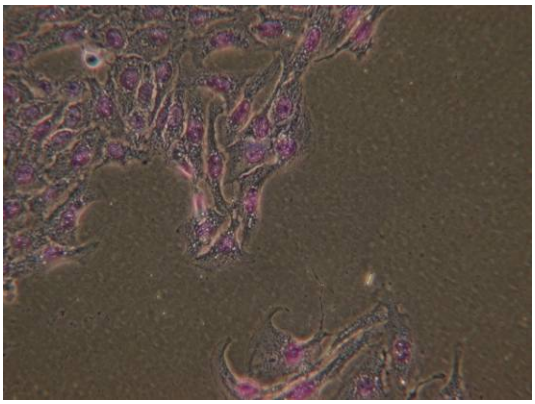
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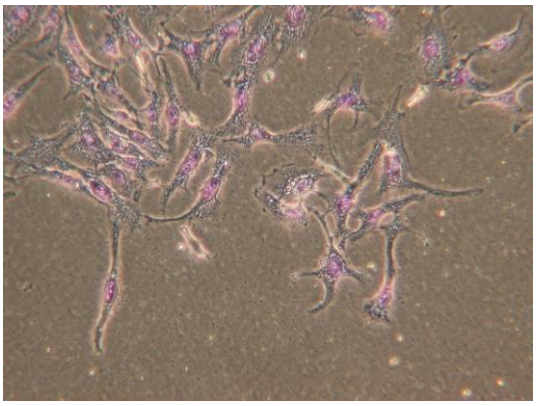


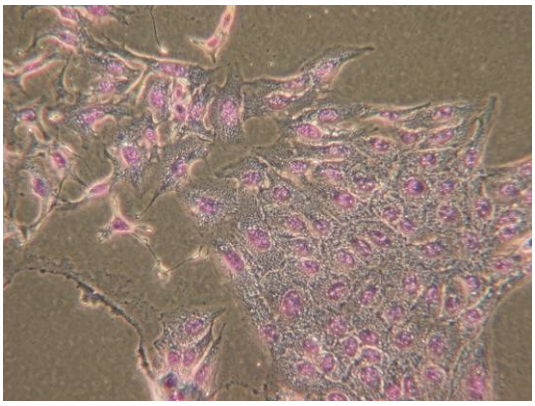


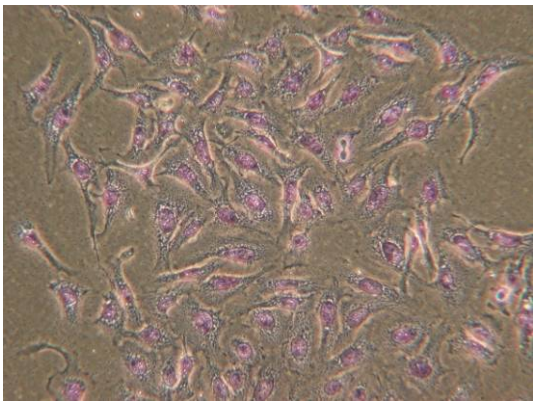






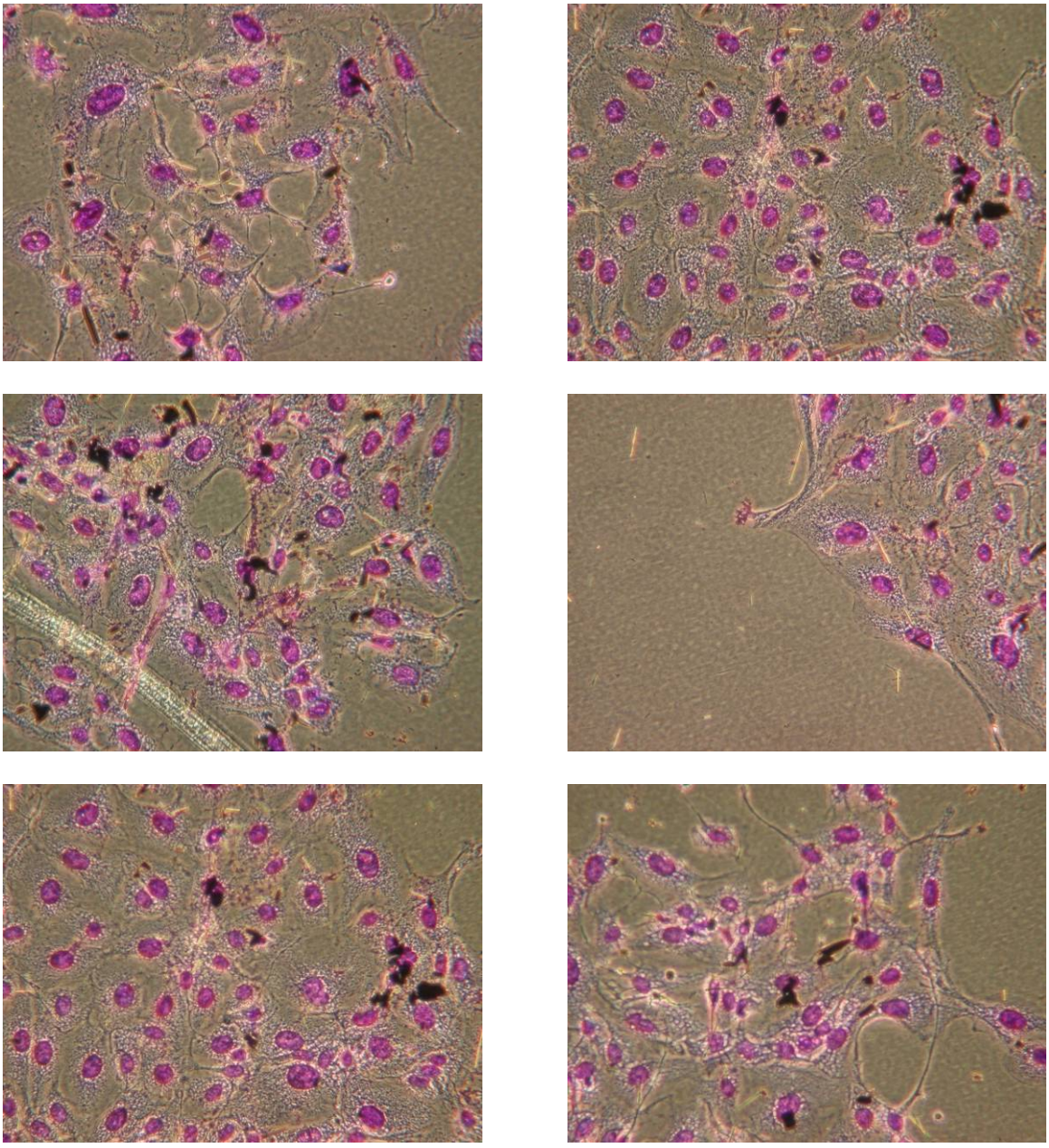


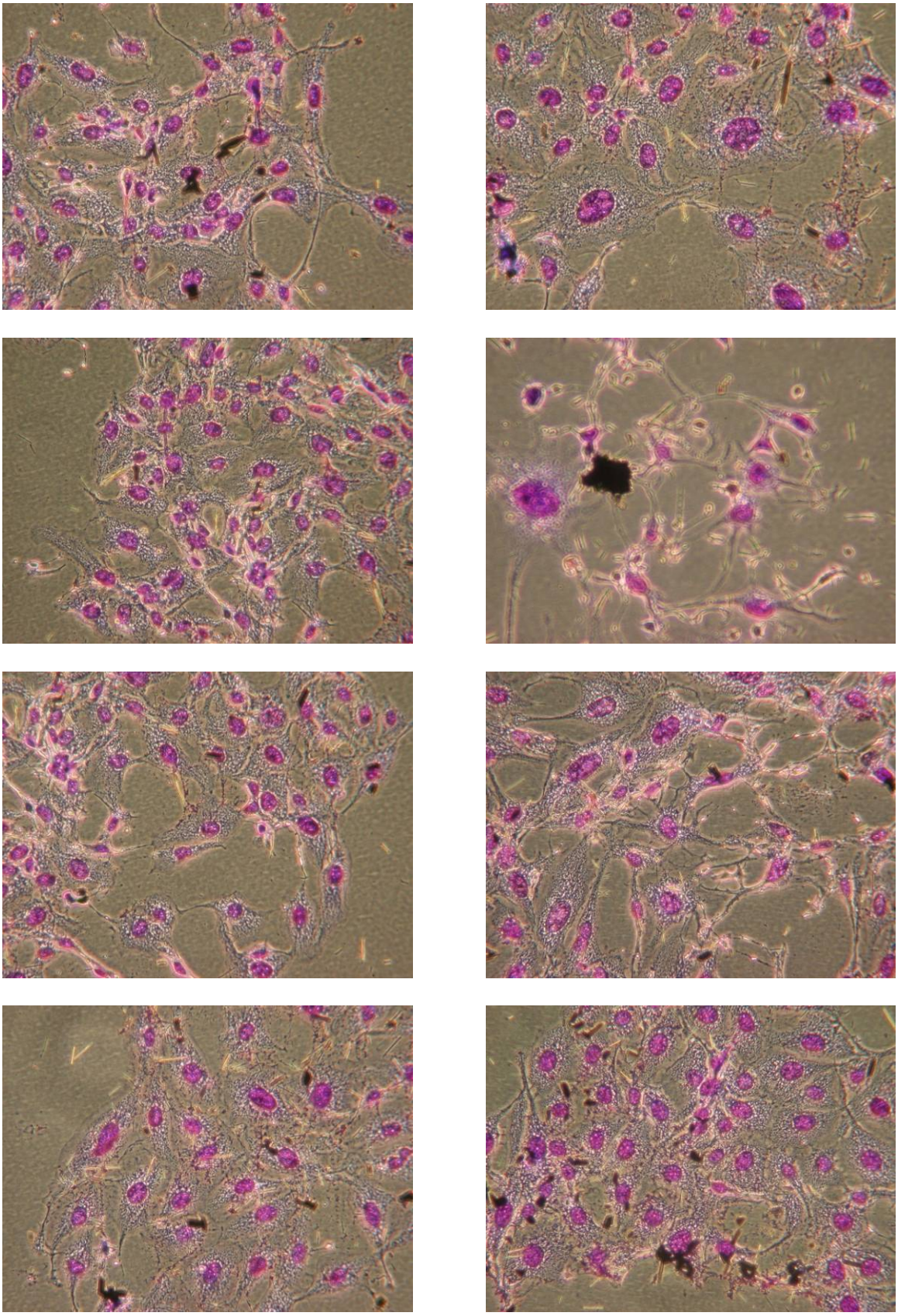


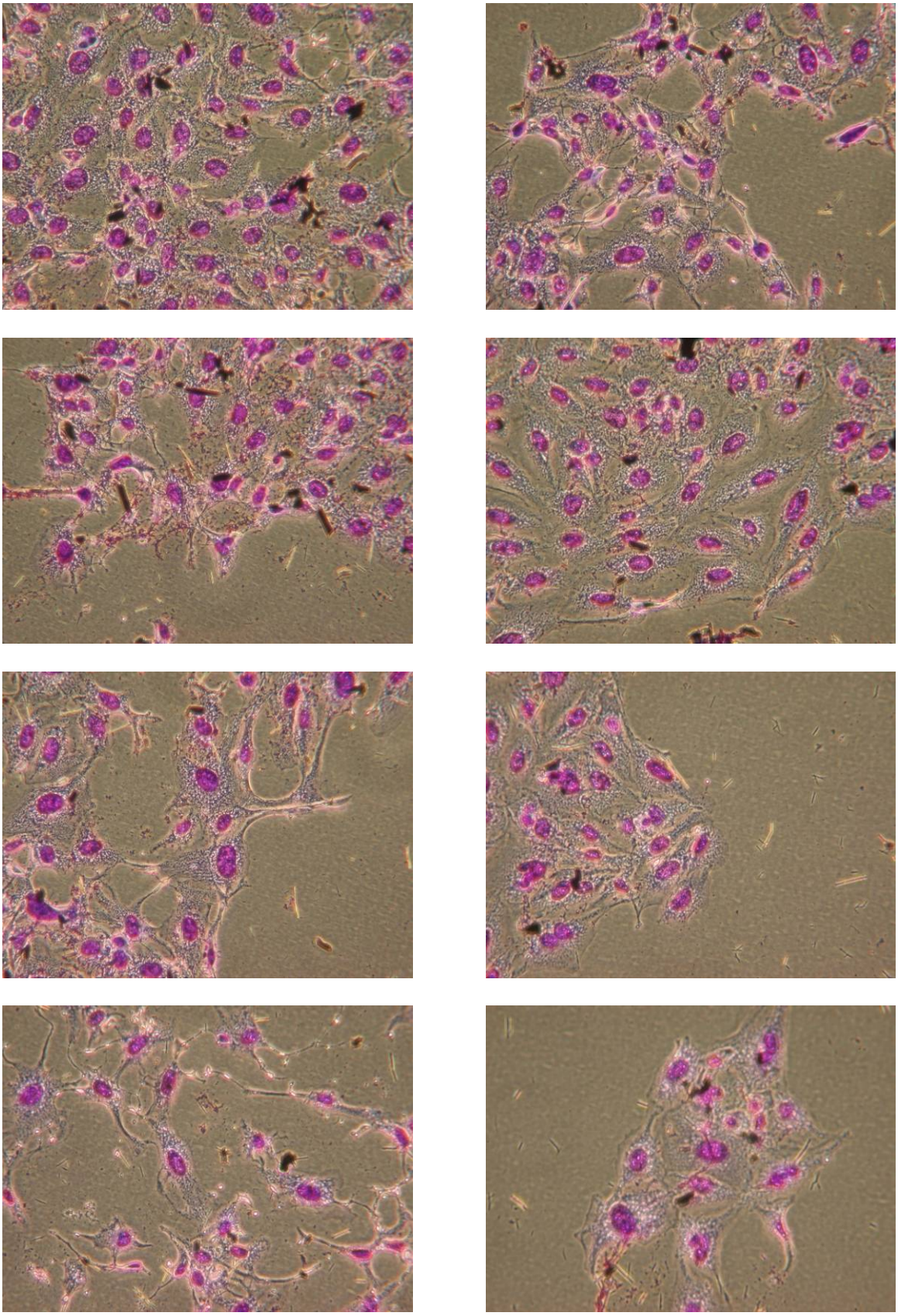


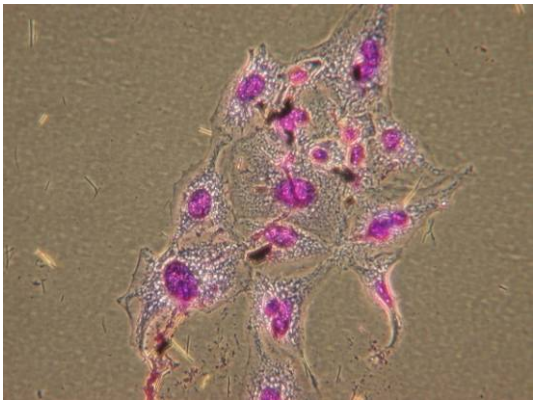
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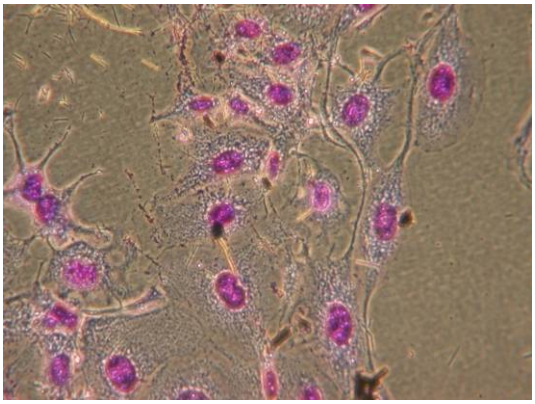
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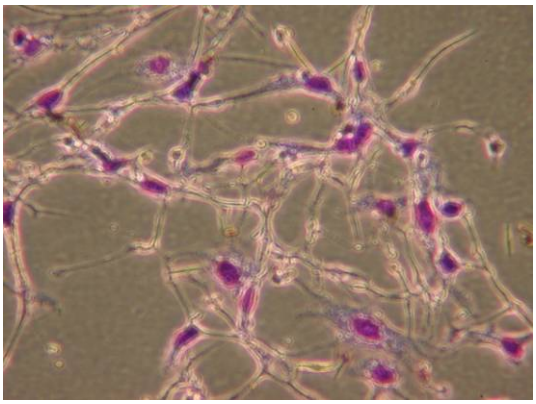


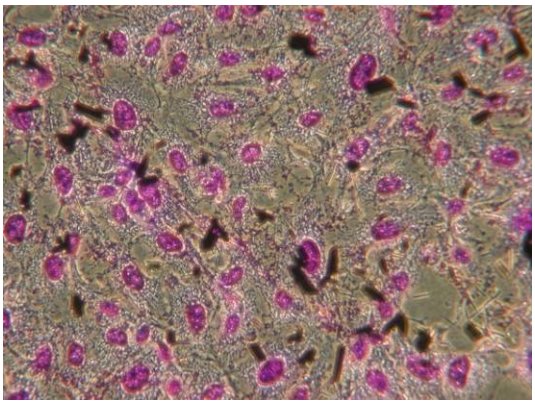


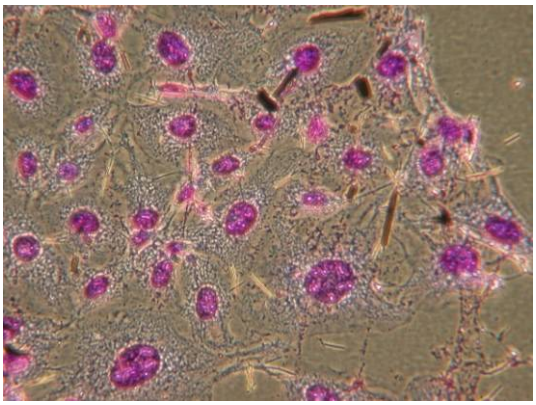


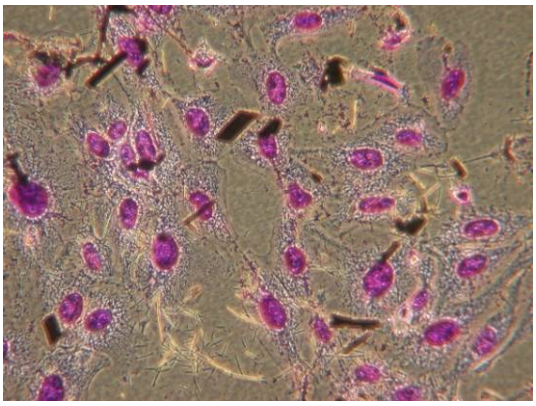






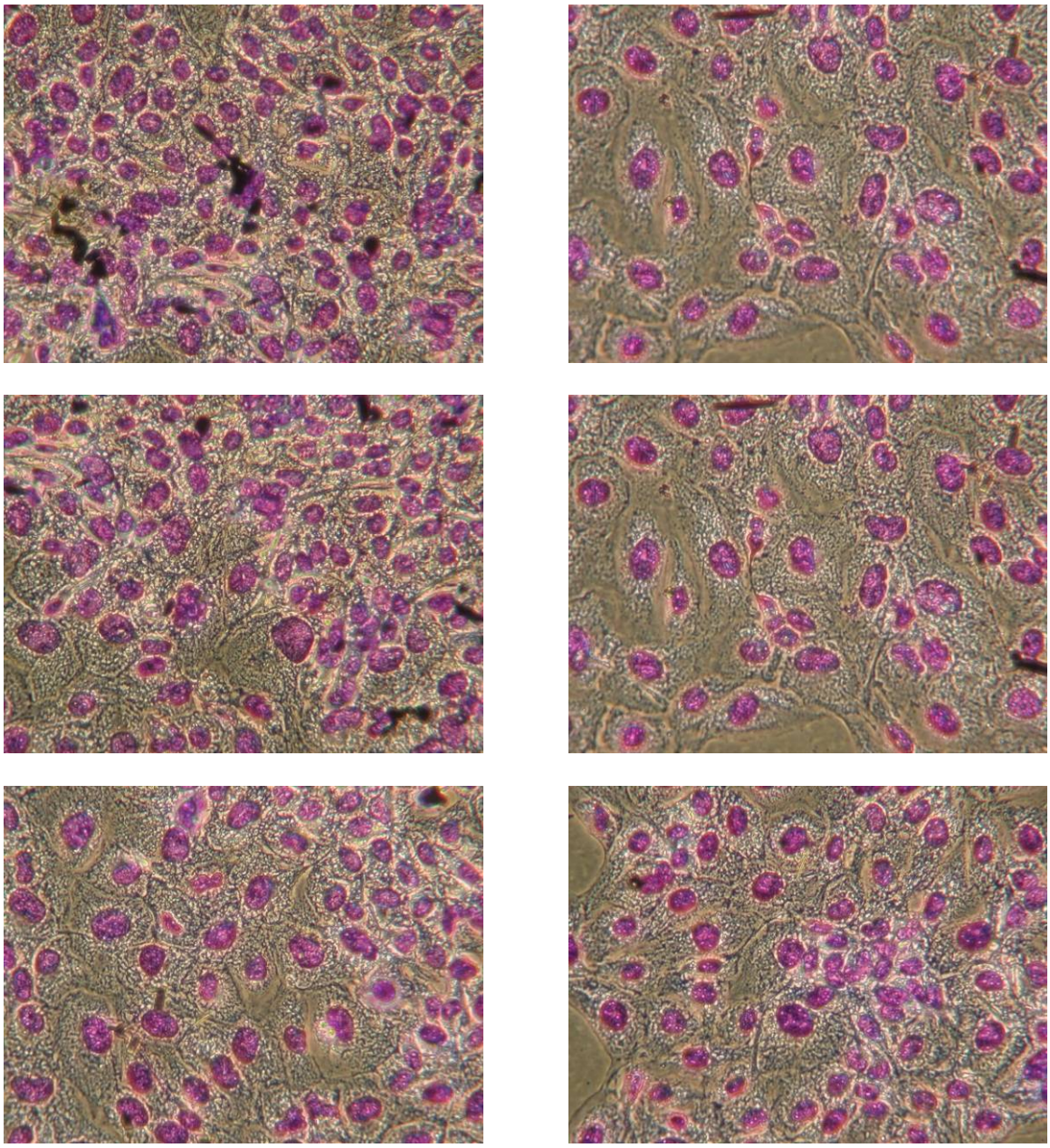


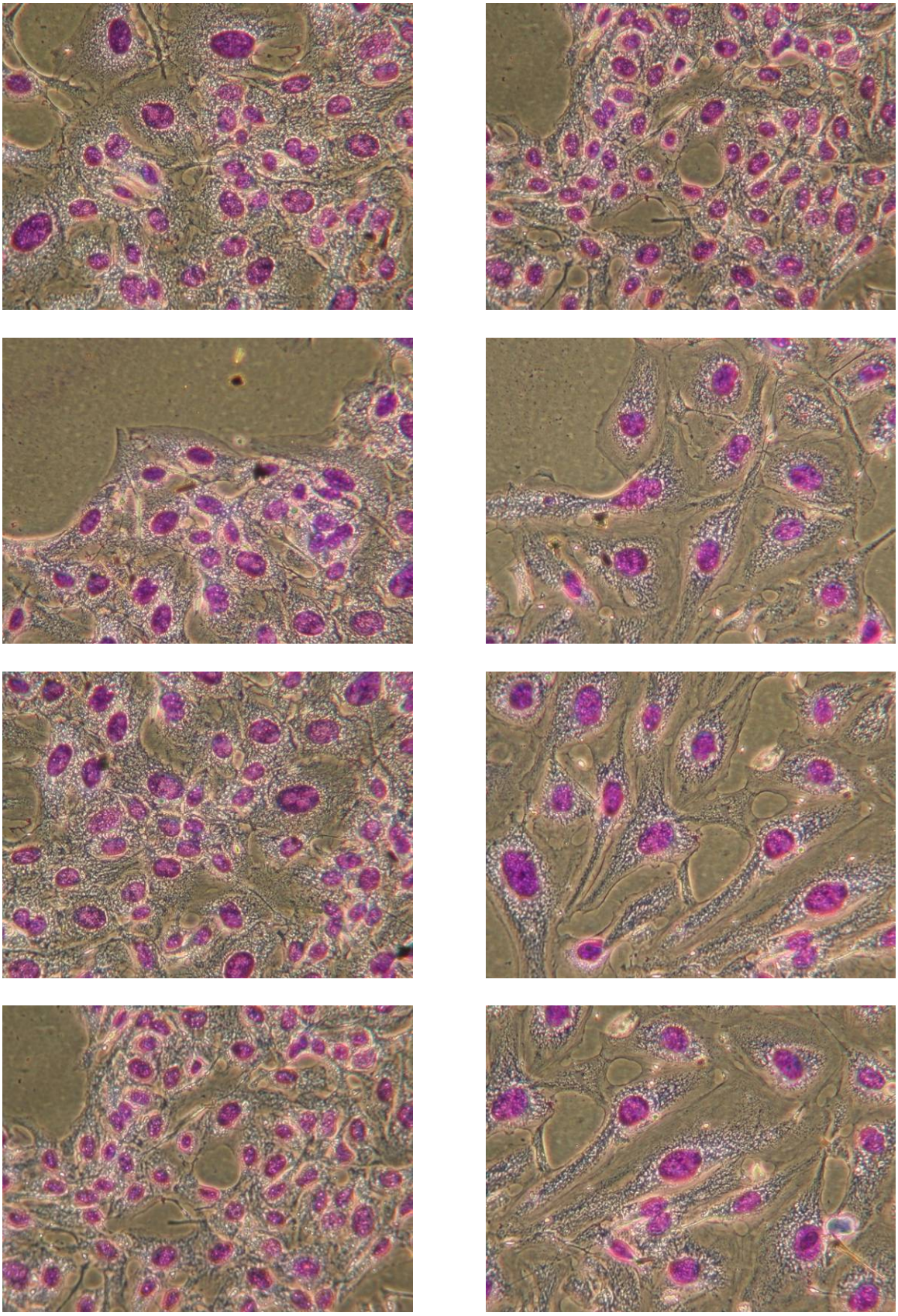


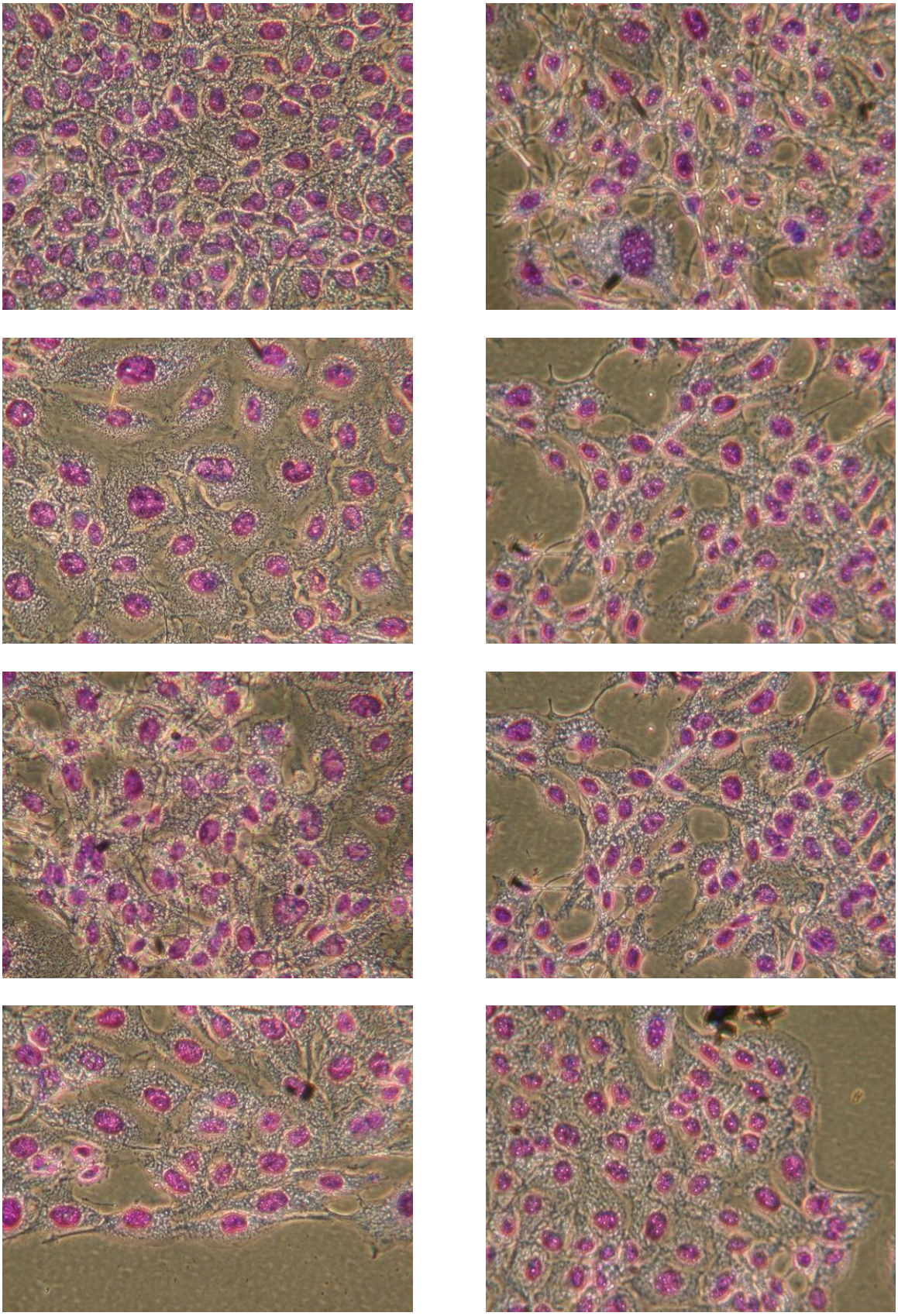


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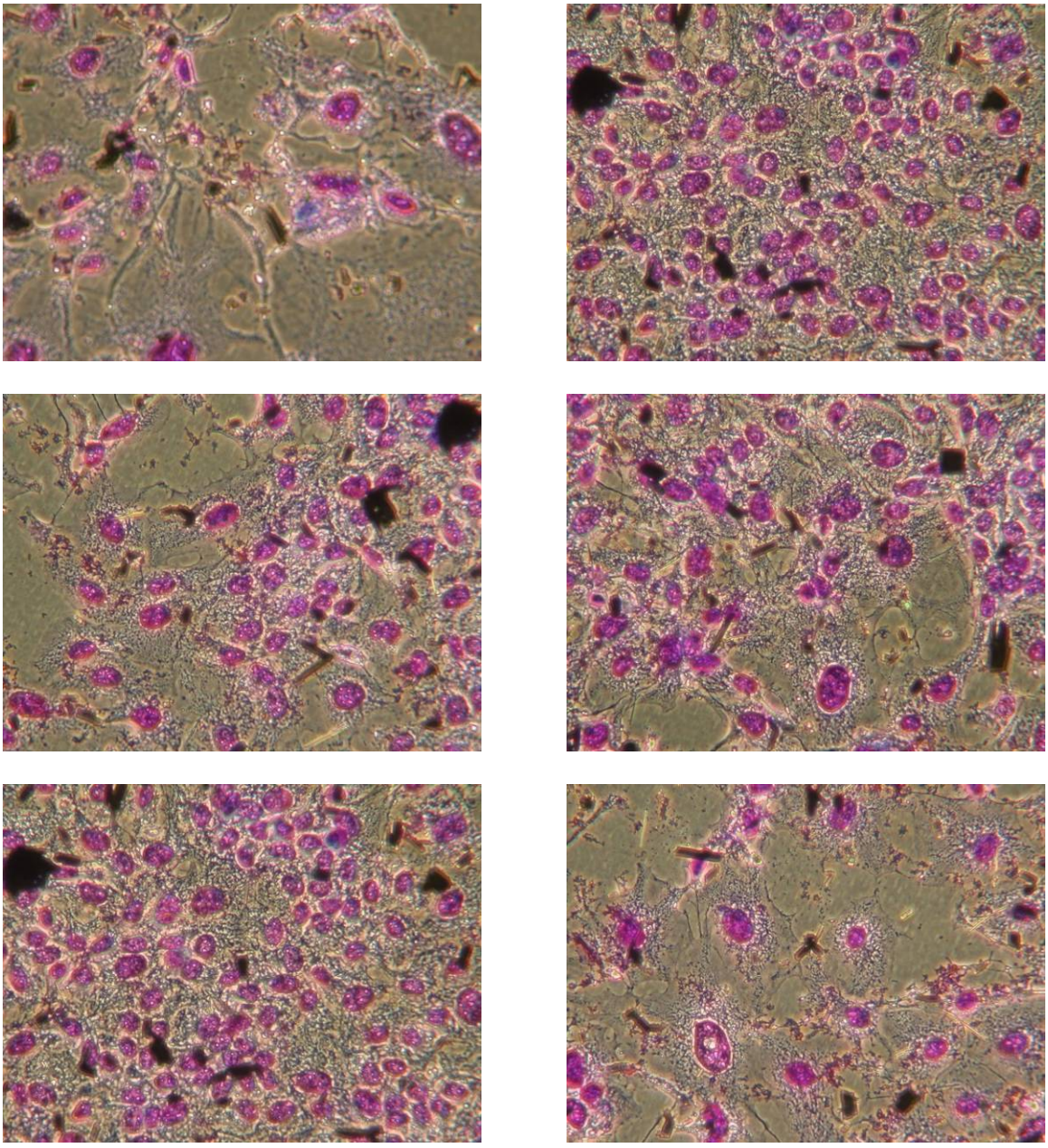


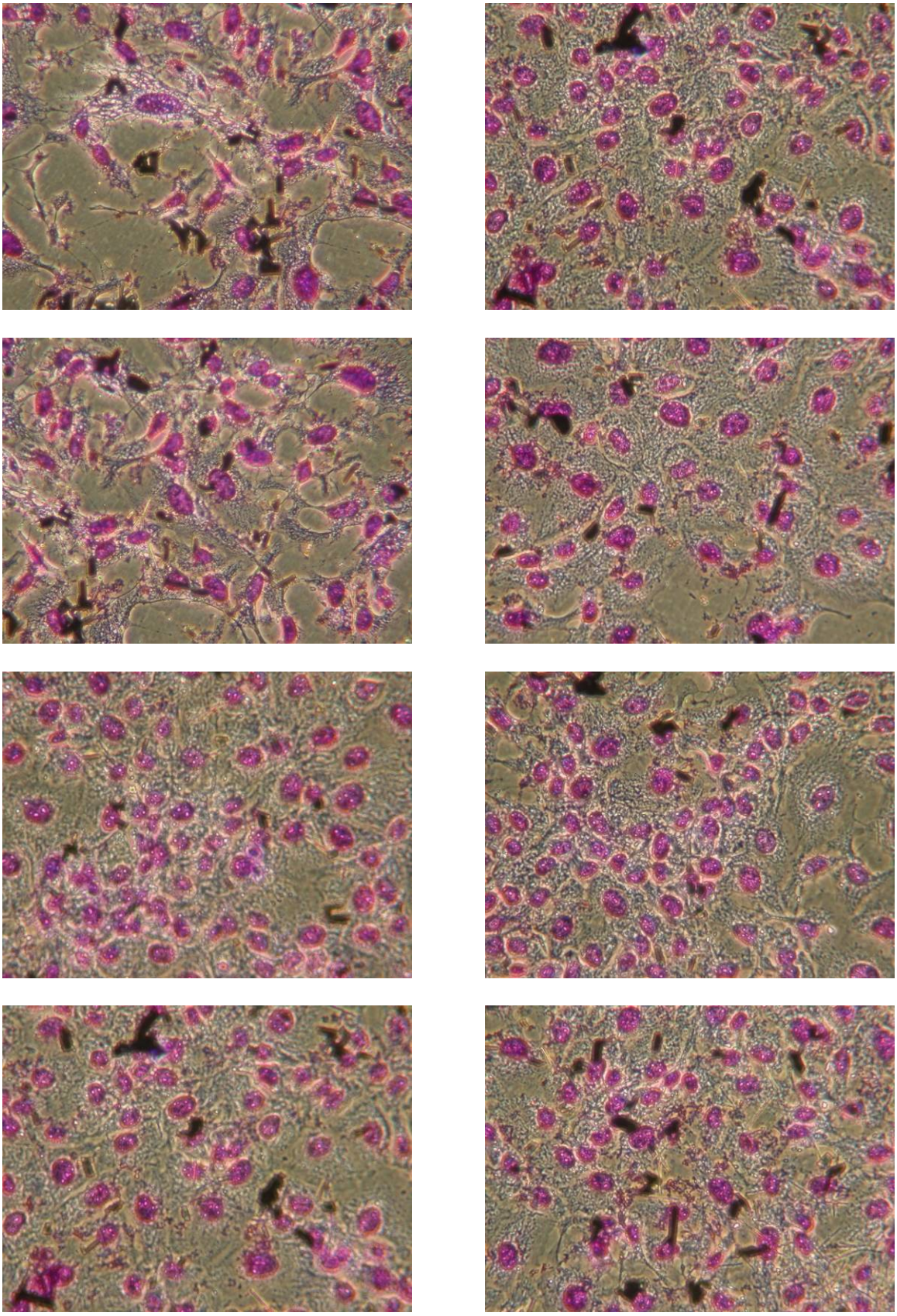


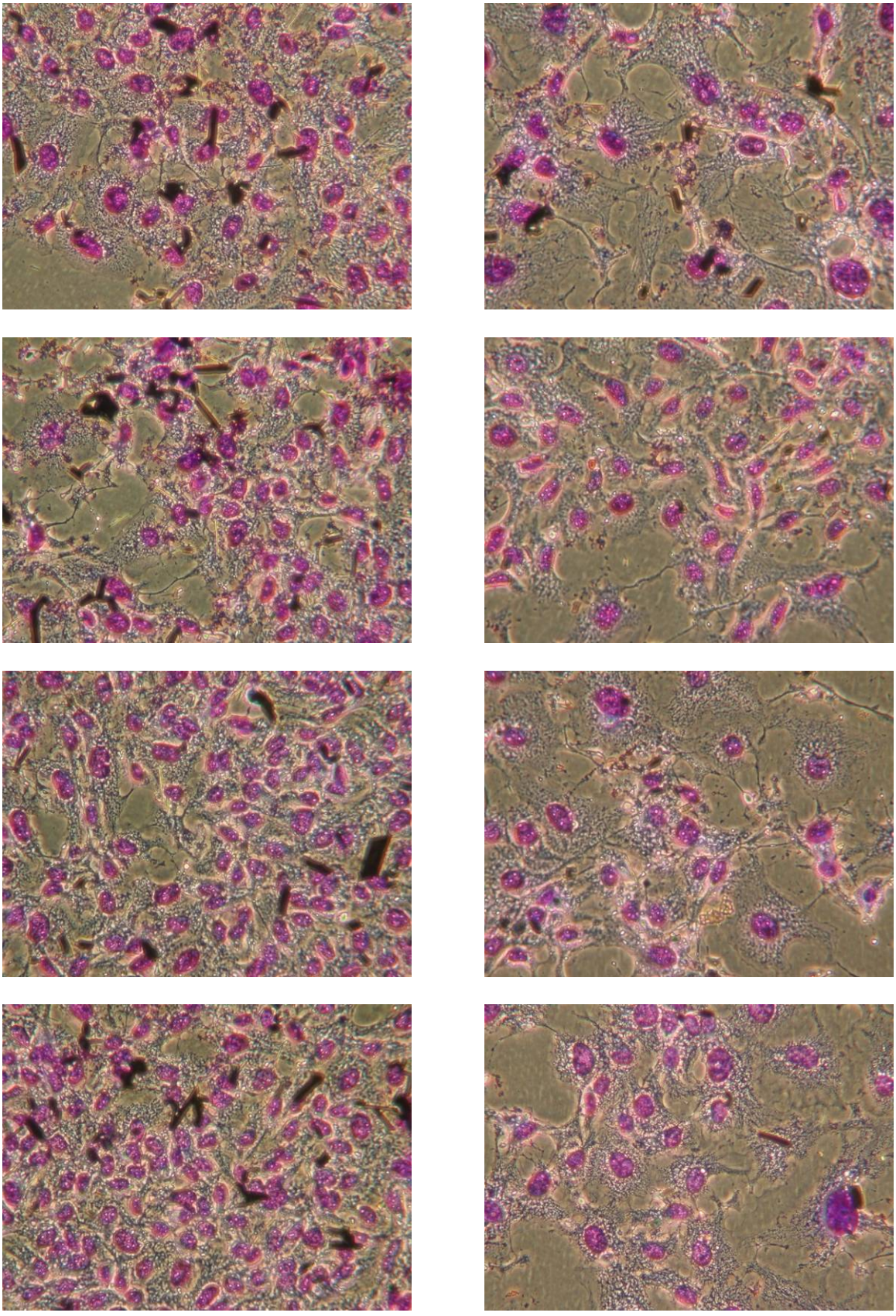


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Appendix B

Provisional Patent Application

TITLE OF INVENTION

Hydrogel for Biomedical Applications

BACKGROUND OF THE INVENTION

1. Field of Invention

[0001] The invention relates to the field of delivery systems for biologically active agents. More specifically, the invention relates to the use of hydrogels for the delivery of biologically active agents.

2. Description of the Related Art

[0002] D-glucaric acid is a phytochemical (plant compound) that occurs naturally in the human body in very small amounts. Several laboratory and animal studies have found that D-glucaric acid seems to have some anti-cancer effects, among other beneficial effects. Many fruits and vegetables contain D-glucaric acid. Additionally, dietary supplements are commercially available that provide D-glucaric acid in the form of a salt, typically calcium D-glucarate.

[0003] Following administration, D-glucaric acid is converted to at least two compounds: D-glucaro-1,4-lactone and D-glucaro-6,3-lactone. Many researchers believe that the former compound (D-glucaro-1,4-lactone) inhibits ~-glucuronidase, an enzyme that has been shown to decrease the rate of elimination of certain hormones and other chemicals from the body, including estrogen and various carcinogens. Accordingly, administration of D-glucaric acid is believed to increase the body's ability to eliminate carcinogens and excess hormones, thereby inhibiting (1) carcinogenesis, (2) malignant tumor growth, (3) metastasis, and (4) tumorigenesis. D-glucaric acid may also interact with signal transduction pathways. Some researchers suggest that D-glucaric acid's anti-cancer effects may arise also from its ability to interact with signal transduction

pathways. Still other researchers suggest that increased concentrations of D-glucaric acid aid in preventing mutations in at least one of the genes *(i.e., ras)* that codes for proteins that stimulate cell growth *(e.g.,* K-Ras). Finally, still other researchers suggest that D-glucaric acid inhibits the ability of some mutagens *(e.g.,* benzo[a]pyrene) to bind to DNA.

[0004] A number of animal studies published in peer-reviewed medical journals have found that, in rats, dietary D-glucaric acid (1) inhibits the development of breast cancer tumors and (2) shrinks some existing tumors. Animal studies also found that dietary D-glucaric acid slowed the development of tumors in the colon, lung, liver, skin, and prostate.

[0005] Other beneficial effects of D-glucaric acid, according to some researchers, include (1) increasing hepatic circulation, (2) decreasing lipid levels in the blood, (3) reducing cholesterol production, and (4) lowering estrogen levels. For example, preliminary human studies have shown that D-glucaric acid reduces total serum cholesterol up to 12 percent, low-density lipoprotein (LDL) up to 28 percent, triglycerides up to 43 percent. There are no known side effects associated with the use of D-glucaric acid.

[0006] In September, 2006, a scientific research article disclosed that D-glucaric acid lactones can readily be grafted onto primary positions of polyolsor polysaccharides under mild non-toxic conditions. c.-L. Davey, *et al., Esterification of select polyols with D-glucaric acid as model reactions for esterification of starch,* Carbohydrate Research, Vol. 341, (2006), pp. 2688-2693. The objective of the research performed by Davey *et al.* was to determine to what extent some select polyols, as structural models for starch, could be directly esterified with D-glucaric acid, which (in aqueous solution) is in equilibrium as the acyclic diacid and the 1,4- and 6,3-D-glucaro lactones. *Id.,* 2688.

Davey *et al.* concluded that transesterification of D-glucaric acid lactones occurs readily

to unhindered primary hydroxyls of polyols and that the pendant D-glucaric acid groups are in the appropriate lactone form rather than in the open-chain acid (acyclic) form. *Id.*, 2689. Accordingly, those pendant lactones can further trans esterify to form linkages between different polyol molecules. *Id.*

[0007] Although many medicines and other biologically active agents currently are available in a controlled-release formulation, there is no known controlled-release formulation for D-glucaric acid. It would be advantageous if D-glucaric acid were available in a controlled-release formulation. It would be advantageous also if such a formulation could be administered parenterally such that a specific location in the body could be targeted for treatment with D-glucaric acid.

BRIEF SUMMARY OF THE INVENTION

[0008] A hydrogel for biomedical applications is disclosed herein. The hydrogel comprises a dispersed phase and a dispersion medium, the latter (dispersion medium) consisting substantially of water. In all embodiments disclosed herein, the dispersed phase includes a polymeric network having both hydrophilic properties and hydrophobic properties. In some embodiments, for example, the polymeric network includes a hydrolyzable starch-derived polymeric substance and a cross-link that has the potential to cause a biological effect following the controlled release *in vivo* of the cross-link from the hydrogel. In some of these embodiments, the cross-link includes D-glucaric acid, a derivative of D-glucaric acid, or an analogue of D-glucaric acid. Among other things, a method is disclosed for increasing the concentration of a biologically active form of D-glucaric acid in a warm-blooded organism.

DESCRIPTION OF THE INVENTION

[0009] The present invention, *i.e.,* a hydrogel for biomedical applications, is described more fully hereinafter. From the outset, it is worth noting that this invention may be embodied in many different forms and should not be construed as limited to the specific embodiments described herein. Additionally, the inventor's thesis titled, "The Synthesis and Characterization of a Novel Polysaccharide Hydrogel for Biomedical Applications," which as of yet is unpublished, is attached hereto as Appendix A and is incorporated into this specification.

[0010] Definitions

[0011] " Analogue" is a chemical compound with a structure similar to that of another but differing from it in respect to a certain component; it may have a similar or opposite action metabolically.

[0012] "Biopolymer" is a polymeric substance (as a protein or polysaccharide) formed in a biological system.

[0013] "Complex" is a molecular entity formed by loose association involving two or more component molecular entities (ionic or uncharged), or the corresponding chemical species. The bonding between the components is normally weaker than a covalent bond.

[0014] "Derive" means to obtain (a chemical substance) actually or theoretically from a parent substance.

[0015] "Parenteral administration" is any route of administration other than the alimentary canal, including, for example, subcutaneous and intramuscular.

[0016] "Polyol" is an alcohol containing more than two hydroxyl groups *(e.g.,* suger alcohols, inositol). Also known as polyhydric alcohol.

[0017] "Polysaccharide" is a carbohydrate that can be decomposed by hydrolysis into two or more molecules of monosaccharides; *especially:* one (as cellulose, starch, or glycogen) containing many monosaccharide units and marked by complexity.

[0018] "Solvation" is any stabilizing interaction of a solute (or solute moiety) and the solvent or a similar interaction of solvent with groups of an insoluble substance. Such interactions generally involve electrostatic forces and van der Waals forces, as well as chemically more specific effects such as hydrogen bond formation.

[0019] "Starch" is anyone of a group of carbohydrates or polysaccharides, of the general composition (C6HlOOS)n, occurring as organized or structural granules of varying size and markings in many plant cells; it hydrolyzes to several forms of dextrin and glucose; its chemical structure is not completely known, but the granules consist of concentric shells containing at least two fractions: an inner portion called amylose, and an outer portion called amylopectin.

[0020] Other definitions will be presented as necessary in the description that follows.

[0021] Hydrogel for Biomedical Applications

[0022] A "hydrogel" is a gel composed usually of one or more polymers suspended in water. It is a colloidal system in which the dispersed phase (colloid) has combined with the dispersion medium (water) to produce a viscous jelly-like product. In all embodiments of the present invention, the colloid includes a polymeric network having both hydrophilic surfaces and hydrophobic surfaces. The hydrophilic surfaces enable the polymeric network to retain water *via* intermolecular forces *(e.g.,* hydrogen

bonding). The hydrophilic surfaces also increase the permeability of the polymeric network to water. In contrast, the hydrophobic surfaces reduce the water solubility of the polymeric network, thus promoting the ability of the polymeric network to interact with water *(i.e.,* the dispersion medium) without being dissolved by it.

[0023] In at least some embodiments of the present invention, the polymeric network is at least partially starch-derived in that it includes a biopolymer that is commonly present in naturally-occurring starch. More specifically, the polymeric network includes amylose and! or amylopectin. Amylose is a polysaccharide composed of unbranched chains of D-glucose units joined by a-l,4'-glycosidic linkages. In contrast, amylopectin is a branched polysaccharide. Like amylose, it is composed of
chains of D-glucose units joined by a-l,4' -glycosidic linkages. Unlike amylose, however, amylopectin also contains a-l,6' -glycosidic linkages, which create branches in the polysaccharide. The ratio of amylose to amylopectin in a starch-derived hydrogel influences the properties of the polymeric network. Accordingly, the performance of
the hydrogel can be altered by changing the ratio of amylose to amylopectin. For example, the susceptibility of the hydrogel to hydrolytic degradation can be altered by adjusting that ratio.

[0024] Another factor affecting performance of a hydrogel is the degree of cross linking in the polymeric network. A "cross-link" is a crosswise connecting part (as an atom or group) that connects parallel chains in a complex chemical molecule *(e.g.,* a polymer). In the majority of instances, a cross-link is a covalent structure, but the term is also used to describe sites of other chemical interactions *(e.g.,* ionic interactions), portions of crystallites, and even physical entanglements. The cross-link.ing of starchderived polymeric networks is generally known in the art, and various methods have been used in making a hydrogel that comprises a cross-linked starch-derived polymeric network. For example, in one method, a mixture including the starch-derived polymeric network and water is heated to at least partially solubilize it (the starch

derived polymeric network). During heating, this polymeric network, which commonly includes amylose and amylopectin, partially unravels. Heating also encourages the helical regions of the polymeric network to uncoil. This unraveling and uncoiling of the polymeric network allows the water in the mixture to more readily

permeate and solvate the network. Additionally, some of the glycosidic linkages in the network are hydrolyzed, further encouraging the unraveling/ uncoiling and, hence, the solvation of the network. As these processes continue, the network is dispersed in the water, thereby increasing the homogeneity of the mixture. After the homogeneity has increased sufficiently, a cross-linking agent is added to the mixture. The heating of the mixture continues, encouraging the cross-linking agent to bond to the polymeric network to yield a cross-linked starch-derived polymeric network. The temperature(s) attained at this stage of heating may vary depending, *inter alia,* on (1) the identity of the cross-linking agent, (2) the concentration of the cross-linking agent in the mixture, and (3) the desired degree of cross-linking in the polymeric network. Thereafter, the mixture is cooled, encouraging the polymer chains in the network to entangle and coil. At least some of the water present in the network upon commencement of the cooling is

retained therein, leading to the formation of the hydrogel.

[0025] In at least some embodiments of the present invention, the hydrogel comprises a polymeric network that is cross-linked. In some of these embodiments, the hydrogel comprises a starch-derived polymeric network that is covalently cross-linked using D-glucaric acid lactone *(e.g.,* D-glucaro-1,4-lactone). For example, the carboxyl

group of D-glucaro-1,4-lactone that includes the C-6 carbon can react with a primary hydroxyl group on a first polymer chain of amylose or amylopectin via an esterification reaction, thereby resulting in a covalent bond between the two. Additionally, the pendant lactone can react with a primary hydroxyl group on a second polymer chain of amylose or amylopectin via a transesterification reaction, resulting in a covalent bond between these two, thereby completing the cross-link. It is worth noting that these ester linkages are susceptible to hydrolysis, as are the glycosidic linkages in amylose,

amylopectin, and other polysaccharides. Accordingly, a hydrogel that comprises a starch-derived polymeric network that is cross-linked via esterification using D-glucaro l,4-lactone is biodegradable, meaning that the hydrogel will erode or degrade *in vivo,* essentially yielding only biocompatible substances, including D-glucaro-l,4-lactone. It is worth noting also that this process can be used in preparing a starch-derived polymeric network that is cross-linked with D-glucaro-6,3-lactone.

[0026] As indicated previously, there is a significant body of evidence tending to establish that D-glucaro-l,4-lactone is a biologically active agent. This body of evidence suggests that D-glucaro-l,4-lactone has anti-cancer effects, among other beneficial biomedical effects. The embodiments of the hydrogel that comprise a starch-derived polymeric network that is cross-linked with D-glucaric acid *(e.g.,* D-glucaro-l,4-lactone) are among those suitable for parenteral administration to a warm-blooded organism. Such embodiments of the hydrogel can be used to increase the concentration of Dglucaric acid in the bloodstream. Furthermore, because such embodiments can be administered parenterally, they facilitate the selective targeting of a location in the organism such that, at least initially, the concentration of D-glucaric acid in that location can be increased substantially relative to the overall concentration of D-glucaric acid in the organism. Thus, for example, a hydrogel comprising a starch-derived polymeric network that is cross-linked with D-glucaric acid can be parenterally administered to a location proximate to a malignant tumor.

[0027] In still other embodiments of the present invention, the hydrogel comprises a starch-derived polymeric network that is cross-linked with a biologically active form of D-glucaric acid *(e.g.,* D-glucaro-l,4-lactone), the cross-link serves at least two purposes. In addition to causing a biological effect upon release from the hydrogel, such a cross-link alters the hydrogel's degradation properties. Accordingly, the degree of cross-linking affects the release kinetics of the biologically active form of D-glucaric acid. A suitable degree of cross-linking can, for example, increase the reliability of the

performance of the hydrogel insofar as release kinetics are concerned. It is worth noting that, in this instance, the degree of cross-linking acquires even greater significance because it is directly related to dosage. Other factors affecting release kinetics include (1) the ratio of amylose to amylopectin (as indicated previously) and (2) the density of the hydrogel. Suitable formulations will be determined by one skilled in the art and will vary depending, *inter alia,* on (1) the route of administration, (2) the desired effect *(e.g.,* anti-cancer), and (3) the desired release kinetics *(e.g.,* sustained release and other types of controlled release). Such formulations may vary also depending on whether the desired biological effect is therapeutic, prophylactic, or diagnostic.

[0028] In still other embodiments of the present invention, the dispersed phase (colloid) of the hydrogel includes at least three different chemical substances: (1) a starch-derived polymeric network, (2) a cross-link, and (3) a biologically active agent. The starch-derived polymeric network is cross-linked to facilitate the controlled release *in vivo* of the biologically active agent. In at least some of these embodiments, the starch-derived polymeric network is cross-linked also to facilitate the retention *in vitro* of the biologically active agent. For example, the cross-link can change the mechanical properties of the starch-derived polymeric network, thereby enhancing the ability of the network to retain biologically active agents in some instances. Such changes can affect the helical regions of the starch-derived polymeric network, allowing these regions to more readily accept and retain a biologically active agent *(e.g.,* an antibiotic). It is worth noting that, in embodiments where the biologically active agent has complexed with the helical or other regions of the starch-derived polymeric network, diffusion can be a
significant factor in effecting the release of the biologically active agent from the hydrogel. Thus, one skilled in the art will consider the process of diffusion as well as the degradability of the hydrogel when formulating and designing such a hydrogel.

CLAIMS

Having thus described the aforementioned invention, what is claimed is:

1. A hydrogel for the controlled delivery of a biologically active agent, said hydrogel comprising:

a dispersed phase including a polymeric network, said polymeric network having both hydrophilic properties and hydrophobic properties, said polymeric network including a hydrolyzable polymeric substance and a cross-link, said cross-link having the potential to cause a biological effect following the controlled release *in vivo* of said cross-link from said hydrogel; and

a dispersion medium comprising water.

2. The hydrogel of Claim 1 wherein said hydrolyzable polymeric substance is at least partially derived from starch.

3. The hydrogel of Claim 1 wherein said hydrolyzable polymeric substance is a polyol or a polysaccharide.

4. The hydrogel of Claim 1 wherein said hydrolyzable polymeric substance is selected from the group consisting of amylose, amylopectin, and any combination thereof.

5. The hydrogel of Claim 1 wherein said cross-link includes D-glucaric acid, a derivative of D-glucaric acid, or an analogue of D-glucaric acid.

6. A method of inhibiting B-glucuronidase, said method comprising the step of parenterally administering to a warm-blooded organism a hydrogel, said hydrogel including a polymeric network having both hydrophilic properties and hydrophobic properties, said polymeric network including a hydrolyzable polymeric substance and a

cross-link, said cross-link including D-glucaric acid, a derivative of D-glucaric acid, or an analogue of D-glucaric acid.

7. The method of Claim 6 wherein said hydrolyzable polymeric substance is at least partially derived from starch.

8. The hydrogel of Claim 6 wherein said hydrolyzable polymeric substance is a polyol or a polysaccharide.

9. The hydrogel of Claim 6 wherein said hydrolyzable polymeric substance is selected from the group consisting of amylose, amylopectin, and any combination thereof.

10. A method of increasing the concentration oIa biologically active form of D'" glucaric acid in a warm-blooded organism, said method comprising the step of parenterally administering to the warm-blood organism a hydrogel, said hydrogel including a polymeric network having both hydrophilic properties and hydrophobic properties, said polymeric network including a hydrolyzable polymeric substance and a
cross-link, said cross-link including D-glucaric acid, a derivative of D-glucaric acid, or an analogue of D-glucaric acid.

11. The method of Claim 10 wherein said hydrolyzable polymeric substance is at least partially derived from starch.

12. The hydrogel of Claim 10 wherein said hydrolyzable polymeric substance is a polyol or a polysaccharide.

13. The hydrogel of Claim 10 wherein said hydrolyzable polymeric substance is selected from the group consisting of amylose, amylopectin, and any combination thereof.

ABSTRACT OF THE DISCLOSURE

A hydrogel for biomedical applications is disclosed, as are various methods relating to its use. The hydrogel comprises a dispersed phase and a dispersion medium, the dispersion medium consisting substantially of water. In all embodiments disclosed herein, the dispersed phase includes a polymeric network. In some embodiments, the polymeric network includes a hydrolyzable polymeric substance and a cross-link that has the potential to cause a biological effect following the controlled release *in vivo* of the cross-link from the hydrogel. Among other things, a method is disclosed for using some embodiments of the hydrogel to increase the concentration of a biologically active form of D-glucaric acid in a warm-blooded organism.

Vita

Elizabeth Diana Barker was born in Memphis, TN on December 5, 1980 to loving parents, Louis and Diana Barker. Libby grew up with her brother Jacob in Southaven, MS. She graduated from Southaven High School in May of 1999. She received a Bachelor's of Science degree in Biomedical Engineering from the University of Tennessee in May 2004. She went on to study Polymer Engineering in the department of Materials Science and Engineering at UT, and received her Master of Science Degree in August 2007.

During graduate school Libby has focused her research on biomaterials, specifically on biodegradable hydrogels for drug delivery systems. The University of Tennessee is currently pursuing a patent for her thesis research entitled, "A Novel Biodegradable Hydrogel for Biomedical Applications Including the Treatment of Malignant Tumors and Prevention of Metastatic Disease."

Libby is currently pursuing a PhD in Molecular Therapeutics and Cell Signaling at the University of Tennessee at Memphis in the Interdisciplinary Program in Biomedical Sciences. She plans to continue research on her patent at St. Jude Children's Research Hospital during her PhD studies. She hopes to be a link between science and medicine and to have the opportunity to contribute to the care of others as a research scientist who is dedicated to providing insight and answers that physicians can use to improve the lives of their patients.