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Elucidation of the physicomechanical and *ab initio* quantum energy transitions of a crosslinked PLGA scaffold

Sibongile R. Sibambo[a], Viness Pillay[a,*], Yahya E. Choonara[a], Riaz A. Khan[b], Joe L. Sweet[c]

[a]*University of the Witwatersrand, Department of Pharmacy and Pharmacology, 7 York Road, Johannesburg, Parktown, 2193, South Africa*
[b]*Biomedical Engineering Laboratory, Center for Nano & Biotechnology, Florida State University, 2525 Pottsdamer Street, Tallahassee, FL 32310, USA*
[c]*Department of Natural Sciences, Albany State University, 504 College Drive, Albany, GA 31705, USA*

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Abstract

This study elucidated the *in vitro* physicomechanical transitions of a crosslinked polylactic-*co*-glycolic acid (PLGA) scaffold, utilizing quantum mechanics to compute the *ab initio* energy requirements of a salted-out and subsequently crosslinked PLGA scaffold interacting with simulated physiological fluid, phosphate buffered saline (PBS) (pH 7.4, 37 °C) at a molecular level. Twenty-six salted-out PLGA scaffolds were formulated using a four factor, two centerpoint quadratic Face-Centered Central Composite Design (FCCD). PLGA molecular mass, PLGA concentration, water volume and salting-out reaction time were the dependant formulation variables. Subsequent to PLGA solubilization in dimethyl formamide (DMF), protonated water was added to induce salting-out of PLGA into a scaffolds that were immersed in PBS, oscillated at 100 rpm, and analyzed at pre-determined time intervals for their physicomechanical and *ab initio* quantum energy transitions. Results indicated that the matrix resilience (MR) decreased with longer incubation periods (MR = 35–45%) at day 30. Scaffolds salted-out using higher PLGA concentrations exhibited minimal changes in MR and the matrix ability to absorb energy was found to closely correlate with the scaffold residence time in PBS. Spartan-based *ab initio* quantum energy predictions elucidated the potential scaffold stability from a molecular viewpoint and its suitability for use in rate-modulated drug delivery.

Keywords: Polylactic-*co*-glycolic acid; Crosslinking; Physicomechanical; *Ab initio* quantum mechanics; Drug delivery

1. Introduction

Amid the array of polymeric biomaterials that have been explored for drug delivery and tissue engineering devices, thermoplastic polyesters such as poly-lactic acid (PLA), poly-glycolic acid (PGA), and polylactic-*co*-glycolic acid (PLGA) are renowned for their biocompatibility and biodegradability [1–4]. PLGA is increasingly being utilized for fabricating drug delivery devices and scaffolds for cell seeding in tissue engineering. Structural stability studies on PLGA regarding solvent addition, physicochemical and physicomechanical transitions, and molecular dynamics during *in vitro*/*in vivo* degradation still require further exploration [5–7]. The influence of these factors on application and adaptation to the microenvironment of the tissue/organ is pertinent to the performance of the device [8,9].

A limitation of PLGA is that bulk erosion occurs when exposed to physiological media. Fluid infiltration instigates hydrolytic cleavage of the ester bonds. Each ester bond cleavage generates more carboxyl end groups that catalyze the hydrolytic reaction of other bonds. Partially degraded macromolecules remain insoluble in the surrounding medium and once the molecular mass of partially degraded macromolecules is sufficiently low diffusion begins leading to bulk erosion [10,11].

Modification of PLGA by a salting-out and subsequent crosslinking technique to form a three-dimensional polymeric scaffold under conditions of altering monomeric ratios, molecular mass, crosslinking reagents and salting-out reaction times may result in a polymer with improved

*Corresponding author. Tel.: +27 11 7172274; fax: +27 11 6424355.
E-mail address: viness.pillay@wits.ac.za (V. Pillay).

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degradation rates, physicochemical and physicomechanical properties.

Salting-out confers partial covalent bonding leading to crosslinked PLGA and an increased viscosity of the evolving structure. Polymeric voids resulting from crosslinked interactions are able to constantly engulf fluid molecules in the presence of physiological media. These voids are a result of interactions between polymeric chains and crosslinking ions. A polymeric scaffold whereby the stereochemical configuration provides a void area for ions to bind without obscuring other atoms is assumed to be moderately stable. Restriction of these voids and subsequently fewer hydrated ion clusters within the matrix will result in further stability to the polymeric backbone. When ions are bound externally to voids it may result in increased swelling of the micro-environmental structure with a decrease in quantum energy. Studies by Paulaitis and Pratt [12] have yielded evidence on the dependence of polymeric hydration free energy on the solute size.

The overall textural properties and energy transitions occurring at a molecular level within the scaffold will depend on the extent of crosslinking within the polymeric voids or ions salted-out externally to form the scaffold. The energy absorbed and matrix resilience (MR) behavior is expected to be enhanced with salting-out and subsequent crosslinking of native PLGA to form a scaffold linking the PLGA structural backbone with crosslinking ions. The degree of physicomechanical enhancement may decrease in phosphate buffered saline (PBS) due to cleavage of the crosslinks resulting in chain disentanglement. However, it is assumed that PLGA scaffolds may react slower than hydrogels, depending on the stereochemical configuration. In general, the PLGA chemical-backbone hydrophobicity combined with hydrolytically degradable anhydride crosslinking results in a controllable surface erosion mechanism, with degradation timescales ranging from 48 h to 1 year [13–17].

The resultant molecular dynamics of a salted-out PLGA scaffold and stereochemical configuration of molecules may also lead to pertinent energy transitions within the matrix of the newly formed scaffold at a molecular level. The energy flux of molecular interactions during salting-out may further elucidate the scaffolds final energy of stability. The emerging field of molecular modeling with *ab initio* quantum energy computations aid in elucidating these energy transitions.

This study proposes to explicate the *in vitro* physicomechanical property transitions of a salted-out and subsequently crosslinked PLGA scaffold employing Design of Experiments. Furthermore, the *ab initio* quantum mechanical energy transitions employing PLGA monomeric units and interacting molecules in PBS (pH 7.4, 37 °C) was explored to deduce the *in vitro* stability of the PLGA scaffold at a molecular interaction level.

2. Materials and methods

2.1. Materials

Resomer® grades comprising PLGA with a 50% lactide content and inherent viscosities ranging from 0.16 to 8.2 dl/g were utilized (Boehringer Ingelheim, Ingelheim, Germany). *N*,*N* dimethyl formamide (DMF) was used as a solvent (Rochelle Chemical, Johannesburg, South Africa) and disodium hydrogen orthophosphate, sodium chloride and potassium dihydrogen phosphate were used to prepare the PBS (Saarchem (Pty) Ltd., Brakpan, South Africa). All other reagents were of analytical grade and used as supplied.

2.2. Formulation of the salted-out PLGA scaffold

PLGA of various molecular masses designated as 1, 2 and 3 in Table 1 were weighed, dissolved in DMF, and placed in 200 mL glass beakers. Varying quantities of protonated water (pH 1.5) (H_3O^+) was added to the polymeric solution and induced salting-out into scaffolds that were vacuum dried to remove excess solvent. The dehydrated scaffold samples were then immersed in 100 mL PBS (pH 7.4, 37 °C) and oscillated at 100 rpm in a shaker bath (Stuart LABEX SBS40). At 0, 7, 10, 26 and 30 days post-incubation the scaffolds were assessed for their physicomechanical properties.

2.3. Construction of the experimental design

Table 1 lists the normalized factor levels for the independent formulation variables. A Face-Centered Central Composite Design (FCCD) was selected for optimization of the PLGA scaffolds. The statistical model allows for simultaneously studying the effect of several independent formulation variables influencing the desired responses, by altering the variables in a limited number of experiments. FCCD was employed in this study to determine coefficients of a second order. This is more superior to conventional methods of optimization which involves varying one factor at a time, while keeping constant all other parameters, thus not screening the main interactions and effects of all the involved factors simultaneously [15] (Table 2).

Table 1
Normalized factor levels of the independent formulation variables for the FCCD

Independent variables	Factor level			Units
	Low	Middle	Upper	
Water volume	10	55	100	mL
PLGA molecular mass[a]	1	2	3	Da
PLGA concentration	1	5.5	10	% w/v
Salting-out reaction time	2	13	24	h

[a]PLGA molecular mass: 1 = 55,000 Da; 2 = 100,000 Da; 3 = 160,000 Da.