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Design and mechanistic evaluation of novel pore-regulated polymer matrices for transmucosal drug delivery

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APPENDICES

APPENDIX A, B AND C

APPENDIX A: ABSTRACT OF JOURNAL PUBLICATION

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Construction and *in vitro* characterization of an optimized porosity-enabled amalgamated matrix for sustained transbuccal drug delivery

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ABSTRACT

This research focused on constructing and characterizing an optimized porosity-enabled amalgamated matrix (P-EAM) for sustained transbuccal drug delivery. An interphase, co-particulate, co-solvent, homogenization technique and lyophilization guided through a Box–Behnken experimental design was employed in the fabrication, characterization and optimization of 15 P-EAMs. The effects of varying factor levels on the characteristic *in vitro* physicochemical performances of the P-EAMs were explored. Formulations had an average weight of 128.44 ± 3.48 mg with a dimensional size of 8 mm by 5 mm. Surface morphology showed varieties of pore structures, widespread distributions and uneven interconnectors. Satisfactory drug-loading was achieved (53.14 ± 2.19–99.02 ± 0.74%). Overall amount of drug released in 8 h was measured by the MDT_{ERR} value which ranged between 22.50 and 225.00 min. Formulation demonstrated significant levels of *ex vivo* bioadhesive strength measured as detachment force (F_{det} = 0.964 ± 0.015 to 1.042 ± 0.025 N) and work of adhesion (w_{adh} = 0.0014 ± 0.00005 to 0.0028 ± 0.00008 J). The potential of the P-EAMs to initiate and sustain *ex vivo* transbuccal permeation of drug was shown and measured as a cumulative value of between 25.02 ± 0.85 and 82.21 ± 0.57% in 8 h. Formulations were mesoporous in nature with pore sizes ranging from 40 to 100 Å characterized by the presence of interconnectors. Statistical constraints were simultaneously set to obtain levels of independent variables that optimized the P-EAM formulation.

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1. Introduction

Porous matrices can be described as those possessing characteristic pore (hole) and interconnecting structures which have a significant influence on their performance. They have been studied over the years for their drug delivery applications and they continue to attract great research interest as they possess attractive features such as: (i) stable configuration, (ii) high surface area, (iii) flexible pore sizes, arranged in various distribution patterns, and (iv) defined surface properties, which are due to their unique porous structural configurations. They have found potential biomedical and drug delivery applications such as in the fabrication of biological tissue scaffolds (Kim et al., 2004; Sohier et al., 2006), implants (Moon Suk et al., 2005), hydrogels (Tang et al., 2005), ceramics (Netz et al., 2005; Miao et al., 2004; Rodriguez-Lorenzo and Ferreira, 2004), biocomposites (Wang et al., 2007), sponges (Portero et al., 2007), microcapsules (Chu et al., 2004), wafers (Bromberg et al., 2001; Matthews et al., 2005; Patel et al., 2007), membranes (Park et al., 1997; Åkerman et al., 1998), nanoparticles (Li et al., 2004) as well as in biomaterials engineering, life sciences and other relevant scientific spheres (Hoa et al., 2006). The above-mentioned qualities provide them with the potential to adsorb/load drug molecules and release them in a reproducible and predictable manner; enhance bio-adhesion to mucosal sites as well as augment permeation for the systemic delivery of drug molecules, which would be specifically useful for drug delivery via the transbuccal route, i.e. via the buccal mucosa (Sher et al., 2007; Zhang et al., 2007). In recent years, the demand for such sophisticated approaches for the delivery of therapeutic agents is on the increase (Tao and Desai, 2003). The particular focus on porous systems for transbuccal delivery lies in the potential use of these systems as inexpensive devices for the release of drugs at controlled, and perhaps time-independent rates. As investigated by Korsmeyer et al. (1983) for a hydrophilic porous system, progressive swelling of the polymer particles occur, emanating in considerable structural changes, which include alteration of the mobility of the macromolecular chains, macromolecular relaxations, and changes of the porous structure (e.g. alteration of the shape and size distribution of the pores). These ultimately impact on the porosity and tortuosity of the system during swelling and diffusional release. Finally, as swelling progresses, diffusion of the drug occurs both through the water-filled pores, and through the swollen polymer, which are influenced by the physical structure of the polymer, as well as the thermodynamic interactions between polymer and solute (Korsmeyer et al., 1983). Such a hydrophilic

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APPENDIX B: ABSTRACTS OF CONFERENCE PROCEEDINGS

Appendix B1

A Pore-Regulated Composite Matrix for Prolonged Transmucosal Drug Delivery

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Purpose: To design and evaluate a pore-regulated matrix for prolonged transmucosal drug delivery.
Methods: Interphase homogenization and lyophilization design was employed in fabricating and evaluating 15 pore-regulated matrices in accordance with a Box Behnken experimental design. Physicomechanical and bioadhesive properties of the matrices were evaluated by textural analysis. Fresh porcine buccal mucosa was employed for permeation studies using Franz diffusion cells. Drug loading capacity (*DLC*) was calculated. Drug release analysis was conducted in closed jars containing 25mL simulated saliva (20rpm; 37°C) in a shaking incubator over 8 hours. Surface morphology was evaluated by SEM. *In vivo* assessments were performed utilizing the buccal region of 2 female, large white pigs (20±2kg) were performed.
Results: Mass distribution relative to porosity varied between formulations (121.95±5.08mg). Matrices displayed physicomechanical and bioadhesive strength (1.014±0.016N). SEM showed a variety of pore structures, distributions and interconnections and drug loading of (53.14±5.19-99.12±8.54%) was achieved. Drug release displayed pseudo-zero and first-order trends. The outcomes of *in vivo* animal studies are yet to be established.
Conclusions: Pore-regulated matrices were prepared and studies thus far revealed the potential for application as a prolonged release, transmucosal drug delivery system.

Appendix B2

Influence of formulation variables on the porosity, bioadhesion and drug release characteristics of a pore-regulated matrix

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Introduction
This study investigated the effects of formulation variables namely solute mass ratio and solvent volume ratio on the release characteristics, surface morphology and bioadhesivity of three devices employing phenytoin sodium (PS) as a model drug. Porosity-controlled matrices which differed in terms of the concentrations of pore-forming agent and particulate components were fabricated in order to investigate the influence of varying levels of porosity on the micromechanical and textural behaviour of the system.

Methods
Three drug-loaded porosity-enabled devices (A, B and C) were produced by the process of lyophilization utilizing combinations of biocompatible and biodegradable cellulose, polyacrylic acid and amino-acid based polymers and inorganic excipients as dispersants (different mass ratio) in a polar protic co-solvent system (volume ratio). *Ex vivo* bioadhesivity testing was performed using a Texture Analyzer fitted with a cylindrical (10mm diameter) and fresh porcine buccal mucosa (0.9±0.1mm thick and 2.25cm^2 surface area). Drug loading capacity (DLC) was calculated ($D_a/D_t \times 100$). Drug release analysis was carried out by placing the formulation in a closed jar containing 25mL of simulated saliva, at 20rpm and 37°C over 8 hours in a shaker bath. Surface morphology was evaluated using scanning electron microscopy (20keV, 1000x magnifications).

Results
Formulations A, B and C adhered to the porcine buccal mucosa with different bioadhesive force magnitudes of 0.963±0.01N, 1.014±0.005N and 1.022±0.03N respectively. 58.99±1.01%, 94.51±2.47% and 97.536±1.21% of drug was loaded unto A, B and C. Drug release from the three matrices was in a controlled manner with varying rates over 8hours following a pseudo-zero order pattern with respective $MDT_{50\%}$ values of 104.00±1.45minutes, 109.40±2.01 minutes and 225.00±3.11minutes. SEM showed an extensive, complex internal micro-pore structure. Formulation A displayed spongy interconnections, scanty pore distribution and average pore width of 25µm; Formulation B showed denser pore distribution, circular pores with thin, web-like interconnections and average pore width of 15µm while Formulation C had sparse pore distribution with rigid interconnections, hexagonal pore structures and an average diameter of 20µm. A notable trend was that pore interconnections appeared to play major a role as regards influencing bioadhesion, drug release and drug loading. The spongy interconnections of Formulation A were weakest in terms of these characteristics while Formulation C with rigid interconnections displayed a stronger behaviour.

Conclusions
The distinct flexible characteristics observed with the formulations imply that porosity-enabled devices may be versatile for specialized drug delivery.

Appendix B3

Physicochemical analysis of the formulation approach employed in constructing a porosity-controlled drug delivery system

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Introduction

This study evaluated the absence/existence of chemical transformations utilizing a type of solute-solvent blending with lyophilization.

Methods

15 porosity-controlled drug carriers were lyophilized products of interphase homogenization which formed solute-solvent, multi-component, homogenous blends. The whole process was channeled with an experimental design. The physicochemical stability of the un-lyophilized blends used in preparing the porous carriers were measured in terms of their rheological behaviour as average viscosity (η) and deformation (δ) at a constant shear rate of 250secs^{-1} employing the Modular Advanced Rheometer System equipped with Haake Rheowin data and job software and the rotor C35/1°Titan sensor type (Haake Mars, Thermo Scientific, Waltham, MA, USA). Pre-frozen blends were subjected to lyophilization placing them into a freeze dryer (Bench Top 2K, Virtis, New York, USA) set at -50°C and 0.42mBar for 48 hours. Post lyophilization, produced porous carriers were assessed using the Bruker Optic FTIR Spectrometer (Tensor 27 Spectrometer, Bruker Optics Inc. Billerica, MA, USA) equipped with Opus V6.0 software. Samples were analyzed at wavenumbers ranging from 4000-400cm^{-1}.

Results

Viscosity values ranged between $0.789\times10^4\pm8.89$mPa.s and $8.658\times10^4\pm14.04$mPa.s which was indicative of the different levels of solute and solvent incorporated into preparing the homogenous blend. Deformation can be defined as a measure of irreversible changes due to inter- and intra-molecular chemical transformations which may result from rearrangement of separate particle and/or molecules with the application of an external force. Deformation values ($\delta = 1.509\times10^4\pm5.556$) remained constant for all the formulation blends. This implies that no chemical changes occurred during the process of physical mixing (blending). Furthermore, the stability of the blends even with the application of an external stress was ascertained. The FTIR spectra of each formulation showed a similar pattern to the other with slight variations in their peak heights which indicative of the differences in transmittance values attributable to the variations in the concentration of each component included for the preparation of the carriers. Characteristic vibrational frequencies of the components were identified (N-H: 2900-3080cm^{-1}, C=N: 1617-1633cm^{-1}, C=C: 1511-1535cm^{-1}, C=O: 1600-1900cm^{-1}, O-H: 3100-3300cm^{-1}, C-ONa: 1354-1387cm^{-1} and C=O=C: 3100-3500cm^{-1}.

Conclusions

Homogenization and lyophilization had no irreversible distorting influence on the stability, flexibility and viscoelasticity of the blend. The method is efficient as carriers retained the contributive effects of individual components.

Appendix B4

A bioadhesive porous matrix for prolonged transbuccal delivery of phenytoin sodium through porcine buccal mucosa

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Introduction
This study focused on the development of an *in vivo* porous matrix and qualitatively analyzing its permeation enhancing capabilities, micromechanical and textural behaviour for transbuccal drug delivery using phenytoin sodium (PS) as the model drug.

Methods
Co-particulate, co-solvent homogenization coupled with lyophilization guided through a Box-Behnken experimental design was employed in fabricating of 15 porous PS-loaded matrices. Biocompatible, bioadhesive and permeation enhancing polymers were used. Porosity (ø) was computed for each matrix using a density (ρ) related mathematical expression. A Texture Analyzer equipped with the 2mm flat-tipped and 50m cylindrical probe respectively were used. The micromechanical properties elucidated were the matrix resilience (R_m), matrix displacement (δ_p), matrix strength (ψ) and fracture energy (F_ϵ). *In vivo* permeation was assessed using 2 healthy female, Large White pigs (20±2kg). Formulations were applied to the non-keratinized region of the anaesthetized pig's buccal mucosa within a mucosal pouch sutured using nylon 3/0 sutures to prevent the pig from chewing or swallowing the formulation. Blood samples (10mL) were taken before administering the formulation and thereafter at t_{1hr}, t_{4hr}, t_{6hr} and t_{24hr} once the pig regained consciousness and was stored at -70°C. Pre-frozen plasma was lyophilized and analysed using Fourier Transform Infra Red Spectroscopy (FTIR) at wavenumbers between 4000cm^{-1}-400cm^{-1}.

Results
A linear relationship was observed between the matrix porosity, displacement and fracture energy (R^2=0.98) while an inverse relationship (R^2=0.45) was noted for matrix resilience and strength. Porosity values ranged between 74-83% indicating the high level of porosity. An increase in porosity resulted in larger matrix displacement values (7.73±0.56mm-12.17±0.72mm) and fracture energies (0.02±0.005J-0.06±0.001J) due to an elevation in matrix entropy during probe penetration. The converse was observed for matrix resilience (1.02±0.15%-2.98±0.39%) and strength (3.41±0.77N/mm and 5.55±0.92N/mm). Generally, an increase in the porogen concentrations and a decrease in co-particle levels resulted in higher porosity. Formulations displayed the potential to dissolve through the pig's buccal mucosa as no remnant was observed after removing the mucosal pouch sutures. FTIR spectra revealed the presence of PS in plasma when compared to the baseline at t_{0hr} before drug administration. The centres of activity that spanned over N-H (3050-2925cm^{-1}), C=N (1607-1623cm^{-1}), C=C (1501-1515cm^{-1}) and C-ONa (1384-1367cm^{-1}) bond vibrations were characteristic of PS, were identified in plasma samples at t_{1hr} - t_{24hr}. Furthermore, a range of spectral transformations such as disappearance of C=O, stretching of N-H, C=N, C=C, C-ONa bonds and peak changes <1300cm^{-1} were observed and may attributed to H-bonding between PS and plasma proteins.

Conclusions
The porous matrix demonstrated the capability to enhance the permeation of PS and its applicability to transbuccal drug delivery.

Appendix B5

Design and Characterization of a Pore-Regulated Matrix for Transmucosal Drug Delivery AM-08-02326

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Purpose.

To fabricate a pore-regulated matrix, characterize its physicochemical and physicomechanical properties for applications in transmucosal drug delivery employing phenytoin sodium and porcine buccal mucosa as a model drug and mucosa respectively.

Methods.

The pore-regulated matrix (8×5mm) was produced by lyophilization utilizing combinations of cellulose, polyacrylic acid and amino-acid based polymers. Inorganic excipients were employed as dispersants in a polar protic co-solvent system. A texture analyzer fitted with either a cylindrical or flat-tipped steel probe were used for measuring bioadhesivity and matrix rigidity respectively. Fresh porcine buccal mucosa (0.9±0.1mm thick and 15±1mm in diameter) was employed for the bioadhesivity and permeation tests. Permeation was measured using Franz Diffusion Cells with simulated saliva (pH 6.8; 37°C) and plasma (pH 7.4; 37°C) as the donor and receiving compartments respectively over 8 hours. The flux of drug through the mucosa was then calculated; [J_{drug} =($\Delta ct/\Delta t$)V/S]. Drug-loading capacity (DLC) was calculated (Da/Dt×100). Drug release analysis was performed by immersing the formulation in 25mL simulated saliva in closed 100mL jars, at 20rpm and maintained at 37°C over 8 hours in a shaker bath. Surface morphology was evaluated using SEM (20keV, 200x magnification).

Results.

Formulations adhered to the porcine buccal mucosa (1.03±0.01N). Matrix rigidity was 4.94±0.08N/mm which reflected physicochemical stability and resistance of the matrix to deformation. Permeation of phenytoin through the porcine buccal mucosa into the receiving compartment was evident (J_{drug}=1.40×10^{-4}mg/cm^{2}sec). A DLC value of 94.51±2.47% was achieved from which 88.92±2.83% of phenytoin was released over 8 hours following a pseudo-zero-order pattern. SEM images revealed a complex internal microporous structure with irregular diffusion channels and pore diameters ranging from 20-60μm.

Conclusion.

The investigated physicochemical and physicomechanical properties revealed the potential of the pore-regulated matrix to be applied for prolonged transmucosal drug delivery.

Appendix B6

The influence of pore morphology on the matrix integrity, drug loading and drug release from a porous delivery platform (AM-08-02371)

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Purpose.

To investigate the influence of pore morphology on the matrix integrity, drug loading capacity and drug release from a porous drug delivery platform employing phenytoin sodium as a model drug.

Methods.

Interphase homogenization guided through a Box-Behnken experimental design was employed for the preparation of 15 porous drug delivery platforms. The effect of pore morphology on the matrix integrity, drug loading capacity and drug release behaviour was assessed. Each phenytoin-loaded porous platform (1.2×0.4cm) was formulated from a co-particulate, homogenous polymeric blend using specialized polystyrene moulds and lyophilization. The physicomechanical properties were elucidated in terms of matrix firmness (MF), matrix fracture energy (MF_e) and matrix resilience (MR) utilizing a texture analyzer. The nature of pores and interconnections were evaluated employing SEM. The Drug-Loading Capacity (DLC) was computed using the relationship; $D_0/D_t \times 100$. A closed system containing 25mL of PBS (pH 6.8; 37ºC), placed in a shaker bath set at 20rpm was utilized for drug release studies over 8 hours.

Results.

The individual masses of the 15 formulations averaged 121.95±5.08mg. Mass distribution relative to porosity varied between formulations. The platforms demonstrated a resistance to deformation despite their porous nature (MF values ranged between 3.67±1.1-5.98±0.4Nmm-1; MF_e=0.02±0.005-0.06±0.01J; MR=1.16±0.6-3.35±1%). SEM showed a variety of pore distributions (scattered, dense and aligned) and interconnections (rigid, web-like, thread-like and spongy). The DLC values showed desirable flexibility in drug-loading. A general trend observed was that formulations displaying scattered pore distributions and web-like/spongy interconnections had lower DLC values (51.29±5.19%) as compared to dense/aligned pore distributions and thread-like/rigid interconnections which had higher DLC values (93.22±8.54%). Phenytoin was released over 8 hours in a pseudo-zero-order or first-order manner which was dependant on the pore morphology. A cumulative release of phenytoin ranging between 81.22±5.51-91.48±1.2% was obtained over the 8 hour period.

Conclusion.

The impact of pore morphology on the matrix integrity, drug loading capacity and drug release was established. Results from this study may be promising for the development of porous drug delivery platforms.

Appendix B7

In Vitro and In Vivo Evaluation of a Bioadhesive Porous Composite for the Sustained Transbuccal Delivery of Phenytoin

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Purpose

To prepare and evaluate a bioadhesive, porosity-enabled composite device (PCD) for application as a transbuccal drug delivery system displaying sustained release of phenytoin sodium as a model drug.

Methods

The PCD was produced by interphase homogenization and lyophilization utilizing various polymeric combinations and the model drug phenytoin sodium (PS). *Ex vivo* drug permeation was investigated using Franz diffusion cells with simulated saliva (pH 6.8) and plasma (pH 7.4) at 37°C over 8 hours. A Texture Analyzer fitted with a 10mm cylindrical probe was used to measure the *ex vivo* bioadhesivity of the PCD. Fresh porcine buccal mucosa (0.9±0.1mm) was excised for bioadhesivity and permeation tests. The drug loading capacity was computed and PS release analysis was performed in a closed vessel with 25mL of simulated saliva (20rpm; 37°C) over 8 hours in a shaking incubator. The surface morphology was evaluated by SEM and average pore width was measured with a porosity analyzer. *In vivo* studies were conducted using 5 healthy female Large White pigs (36±3kg) and each formulation was applied to the non-keratinized region of the buccal mucosa and blood was sampled over 24 hours and analyzed by Ultra Performance Liquid Chromatography (UPLC).

Results

PCDs (8×4mm) had an average mass of 128±3.49mg and bioadhered to the buccal mucosa (1.03±0.01N). 89.98±2.34% of drug permeated through the mucosa into simulated plasma. 94.51±2.47% of PS was loaded into the matrix and 90.92±2.83% was released in a controlled manner over 8 hours following pseudo-zero order kinetics. The PCDs were mesoporous (86.01±4.04Å) with an extensive internal pore structure and asymmetrical inter-pore connections. The tested formulations were well tolerated by the pigs indicating that the formulation may be potentially non-toxic. *In vivo* drug release profiles were controlled and biphasic with maximum plasma concentrations of 20.73±5.09µg/mL achieved at t_{6hours}.

Conclusions

In vitro and *in vivo* studies revealed the potential of the PCD to function as a bioadhesive delivery system for the sustained transbuccal release of PS.

Appendix B8

Histomorphological Evaluation of a Mucoadhesive, Porous Matrix for Controlled Transbuccal Drug Delivery

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Purpose

This study focused on designing a mucoadhesive porous matrix, assessing its *ex vivo* drug permeation initiating and sustaining kinetics as well as evaluating the buccal mucosal histology post administration using the pig model.

Methods

The porous matrix was prepared by homogenizing both polymeric and non-polymeric release modifying, permeation enhancing, and mucoadhesive compounds dispersed in a co-solvent system to produce a homogenous blend that was lyophilized. *Ex vivo* drug permeation was investigated using Franz Diffusion Cells with simulated saliva (pH 6.8) and plasma (pH 7.4) at 37°C over 8 hours. A Texture Analyzer fitted with a 10mm cylindrical probe was employed for measuring the *ex vivo* bioadhesivity of the formulation. Fresh porcine buccal mucosa (0.9±0.1mm thick) was employed for the bioadhesivity and permeation tests. Drug loading capacity was computed and the surface morphology was evaluated using SEM and porosity was measured by porositometric analysis. *In vivo* histomorphological studies were conducted using 3 healthy Large White Pigs (36±3kg) and a drug-loaded and placebo formulation was applied to the non-keratinized region of the buccal mucosa. Experimental biopsy samples and their controls, about 3mm deep (1mm^2 area), were taken from the administration site and control sites. The specimens were stored in 10% formalin for analysis. They were processed with routine histological methodology in an automated tissue processor after which tissue blocks were sectioned at 5µm and the slides that were produced stained using haematoxylin and eosin in an automated stainer before evaluation under the electron microscope.

Results and Discussion

The formulation bio-adhered to the buccal mucosa (1.07±0.02N). 90.05±1.15% of carbamazepine was loaded into the matrix and 83.56±3.62% permeated through the porcine tissue in a controlled manner over 8 hours following pseudo-zero kinetics. The formulation was mesoporous in nature (80.12±3.42Å) and presented with a widespread internal spherical pore structure with asymmetrical channels. The pig model employed tolerated the formulation well indicating its non-toxicity. SEM images revealed that the formulation had minimal, non significant pathological effects on the buccal epithelium and lamina propria of the porcine buccal mucosal epithelium. This implied that the polymeric, non-polymeric and active drug components of the porous matrix formulation had no irritable or toxic effects on the porcine buccal mucosa thereby making it potentially suitable for its intended transbuccal delivery application.

Conclusions

The porous matrix displayed the potential to initiate and sustain tissue permeation of drug molecules in a controlled manner for possible transbuccal drug delivery applications.

APPENDIX C: UNIVERSITY OF THE WITWATERSRAND ANIMAL ETHICS CLEARANCE CERTIFICATE

AESC 3

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

STRICTLY CONFIDENTIAL

ANIMAL ETHICS SCREENING COMMITTEE (AESC)

CLEARANCE CERTIFICATE NO. 2007/42/'05

APPLICANT: OA Kolawole

SCHOOL: Pharmacy and Pharmacology
DEPARTMENT:
LOCATION: Medical School

PROJECT TITLE: **An in vivo assessment of novel pore-regulated polymer matrices for oramucosal drug delivery in pigs**

Number and Species

24 Large white pigs < 15kgs; male or female

Approval was given for to the use of animals for the project described above at an AESC meeting held on 20070529. This approval remains valid until 20090529

The use of these animals is subject to AESC guidelines for the use and care of animals, is limited to the procedures described in the application form and to the following additional conditions:

Signed: _____ (Chairperson, AESC) Date: 31/05/07

I am satisfied that the persons listed in this application are competent to perform the procedures therein, in terms of Section 23 (1) (c) of the Veterinary and Para-Veterinary Professions Act (19 of 1982)

Signed: _____ (Registered Veterinarian) Date: 31/05/07

cc: Supervisor: #
Director: CAS

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