Amorphism and polymorphism
of azithromycin

ROELF WILLEM ODENDAAL
(M.Sc, B. PHARM.)

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Supervisor: Prof. W. Liebenberg
Co-supervisor: Dr. M.E. Aucamp
2 KOR. 12:9

“My genade is vir jou genoeg. My krag kom juis tot volle werking wanneer jy swak is.”
Dedicated to my Heavenly Father

AND

my parents, W.T. and Antoinette Odendaal

AND

my late grandparents,

Roelf & Petronella Odendaal

Hans & Lea van Dyk
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LIST OF ABBREVIATIONS

> - greater than
\(\geq\) - greater than or equal to
\(\leq\) - less than or equal to
< - less than
\(\degree\) - degrees
\(\degree\)C - degrees Celsius
\(\Omega\) - ohm

\% - percentage
\% WL - percentage weight loss
\(\theta\) - Theta
\(\mu\)g - microgram
\(\mu\)L - micro litre
\(\mu\)m - micro metre

A - surface area
API - active pharmaceutical ingredient
ATP - adenosine triphosphate
AVG - average
AZM - azithromycin
AZM-DH - azithromycin dihydrate
AZM-G - azithromycin glass
BET - Brunauer-Emmett-Teller theory
BP - British Pharmacopeia
C - carbon

\(C_0\) - initial concentration
cm - centimetre
conc. - concentration

DCM - dichloromethane
DL - limit of detection
DRIFTS - diffuse reflectance infrared Fourier transform spectroscopy
DSC - differential scanning calorimetry
e.g. - *exempli gratia* (for example)
EP - European Pharmacopeia
FTIR - Fourier transform infra-red spectroscopy
g - gram
h - hour
H - hydrogen
H₂O - water
HCl - hydrochloric acid
HPLC - high performance liquid chromatography
HSM - hot stage microscopy
ICH - International Conference on Harmonisation
i.e. - *id est* (that is)
IR - infrared spectroscopy
IUPAC - International Union of Pure and Applied Chemistry
J - Joule
K - Kelvin
KBr - potassium bromide
KCl - potassium chloride
kg - kilogram
KFT - Karl Fischer titration
L - litre
M - molar
m - metre
MAC - *Mycobacterium avium* complex
MDCK - Madin-Darby canine kidney
mg - milligram
MIC - minimum inhibitory concentration
min - minutes
mL - millilitre
mΩ - milli-ohm
MM - molecular mass
mm - millimetre
N - nitrogen
N - newton
NaOH - sodium hydroxide
nm - nanometre
O - oxygen
PAMPA - Parallel artificial membrane permeability assay
P$_{app}$ - apparent permeability coefficient
P-gp - P-glycoprotein
ppm - parts per million
QL - limit of quantification
r$^2$ - correlation coefficient
RH - relative humidity
RNA - ribonucleic acid
rpm - revolutions per minute
RSD - relative standard deviation
s - second
SA - South Africa
SEM - scanning electron microscopy
STDEV (SD) - standard deviation
TAM - Thermal Activity Monitor
TEER - Trans-epithelial electrical resistance
$T_g$ - glass transition temperature
TGA - thermogravimetric analysis
$T_m$ - melting temperature (point)
UFLC - Ultra fast liquid chromatography
USA - United States of America
USP - United States Pharmacopeia
UV - ultraviolet
vs - versus

w/w - weight per weight

XRPD - X-ray powder diffraction
CHAPTER 5: AZITHROMYCIN GLASS

\[ R_L = \frac{1}{1 + b_L C_0} \]  \hspace{1cm} (5.1)

\[ T_{g_{mix}} = w_1 T_{g1} + w_2 T_{g2} \]  \hspace{1cm} (5.2)

\[ T_{g_{mix}} = \frac{(w_1 T_{g1} + k w_2 T_{g2})}{(w_1 + k w_2)} \]  \hspace{1cm} (5.3)

Where \( k = \frac{(p_1 T_{g1})}{(p_2 T_{g2})} \)

\[ \frac{1}{T_{g_{mix}}} = \frac{w_1}{T_{g1}} + \frac{w_2}{T_{g2}} \]  \hspace{1cm} (5.4)

CHAPTER 6: PRODUCT DEVELOPMENT

\[ P_B = w/v \]  \hspace{1cm} (6.1)

\[ P_T = w/v \]  \hspace{1cm} (6.2)

% compressibility = \( \frac{(P_T - P_B)}{P_T} \times 100 \) \hspace{1cm} (6.3)

Hausner ratio = \( P_T / P_B \) \hspace{1cm} (6.4)
CHAPTER 7: PERMEABILITY

\[ P_{\text{app}} = \frac{\text{d}C}{\text{d}t} \times \left( \frac{1}{A \times C_0 \times 60} \right) \] \hspace{1cm} (7.1)

\[ \%_{\text{AZM-G}} = \left( \frac{\text{AZM-G} P_{\text{app}}}{\text{AZM-DH} P_{\text{app}}} \right) \times 100 \%_{\text{AZM-DH}} \] \hspace{1cm} (7.2)

\[ \% \text{ improvement} = \%_{\text{AZM-G}} - 100 \] \hspace{1cm} (7.3)
Azithromycin, an azalide and member of the macrolide group, is a broad spectrum antimicrobial, representing one of the bestselling antimicrobials worldwide. It is derived from erythromycin and exhibits improved acidic stability as a result of its structural modifications. The stable solid form of azithromycin is its dihydrate, although it also naturally occurs in its metastable forms, i.e. the monohydrate and anhydrate. Because azithromycin is poorly soluble in water, its absorption from the gastro-intestinal tract is negatively influenced, which ultimately affects its bioavailability following oral administration (37 %).

Polymorphic (monohydrates and dihydrates) and anhydrous forms of azithromycin were screened and investigated. One anhydrous form also proved to be amorphous, which shifted the focus of this study from polymorphism to amorphism. An amorphous glassy azithromycin was subsequently prepared and fully characterised to present its solid state profile.

The stability of this amorphous glassy form was established at a high temperature and relative humidity over a period of four weeks. Exposure to increased relative humidity (up to 95 %) and increased water content (up to 50 %) also served as stability indicating tests. Its solubility in various aqueous media was determined. A solid dosage form (tablet), containing the azithromycin glass, was prepared, whereafter these tablets were subjected to dissolution studies in different aqueous media. The stability of azithromycin glass in tablet form was determined over a period of three months. The permeability of azithromycin glass across excised pig intestinal tissue was further established at various pH values.

This amorphous glassy form of azithromycin (AZM-G) proved to be very stable at high temperature and relative humidity, whilst also remaining stable after prolonged exposure to 95 % of relative humidity, as it only adsorbed moisture onto its surface. Water content (up to 50 %) had no plasticising effect on azithromycin glass. It demonstrated a significantly higher water solubility (339 % improvement) in comparison with the commercially available azithromycin dihydrate and was it also 39 % more soluble in phosphate buffer (pH 6.8) than its dihydrate counterpart.
The prepared azithromycin glass tablets showed a promising dissolution profile in water, due to the improved water solubility of this glass form. The transport of azithromycin glass at higher pH values (6.8 and 7.2) across the membrane proved to be significantly higher than that of azithromycin dihydrate, thus also illustrating its pH dependence for its transport across pig intestinal tissue.

The improved water solubility of the azithromycin glass, together with its faster dissolution rate, its superior stability and its increased permeability, may ultimately result in a higher azithromycin bioavailability following oral administration.

These research outcomes hence give rise to the need for investigating the effect of administering lower dosages of azithromycin and to determine whether the same antimicrobial efficacy would possibly be achieved, due to maintaining the same tissue concentration levels at these lower dosages.

**Key words:** Macrolide; Azithromycin; Amorphous; Solubility; Stability; Dissolution; Permeability.
UITTREKSELT

Asitromisien, ‘n asalied wat deel van die makroliedgroep vorm, is ‘n breë sprektuur antimikrobie geneesmiddel, wat wêreldwyd as een van die topverkopers van antimikrobiese geneesmiddels beskou word. Asitromisien is ‘n derivaat van eritromisien en toon verbeterde stabiliteit in suur omgewings, as gevolg van sy structurele modificasies. Die dihidraat word as die stabiele soliede vorm van asitromisien beskou, alhoewel dit ook natuurlik in sy monohidraat- en anhidraat vorms voorkom. Omdat asitromisien swak oplosbaar is in water, word sy absorpsie vanuit die gastroïntestinale kanaal negatief beïnvloed, wat uiteindelik weer sy biobeskikbaarheid na orale toediening effekteer (37 %).

Polimorfiese (monohidrate en dihidrate) en anhidriese vorme van asitromisien is oorsigtelik tydens hierdie studie ondersoek. Een anhidraat het ook amorfie eienskappe getoon, wat daartoe aanleiding gegee het dat die fokus van hierdie studie vanaf polimorfisme na amorfisme verskuif het. ‘n Amorfe glas van asitromisien is vervolgens berei en volledig in terme van vaste vorm eienskappe gekarakteriseer.

Die stabilitate van die bereide amorfe asitromisien was by toestande van hoë temperatuur en relatiewe humiditeit oor ‘n tydperk van vier weke bepaal. Blootstelling aan verhoogde relatiewe humiditeit (tot en met 95 %) en verhoogde waterinhoud het voorts as stabiliteitsaanduidende toetse gedien. Die oplosbaarheid van die glas is ook in verskeie waterige mediums bepaal. Amorfe asitromisien is vervolgens in ‘n tabletvorm geformuleer, waarna dissolusie studies op die tablette in verskeie waterige mediums uitgevoer is. Die stabilitate van amorfe asitromisien in tabletvorm is oor ‘n tydperk van drie maande bepaal. Die deurlaatbaarheid van amorfe asitromisien oor uitgesnyde intestinale varkweefsel by verskillende pH-waardes is verder ook bepaal.

Hierdie amorfe, glasagtige vorm van asitromisien het bewys gelewer dat dit baie stabiel was, ten spyte van blootstelling aan beide hoë temperatuur en relatiewe humiditeit. Verlengte blootstelling aan 95 % relatiewe humiditeit het ook geen effek op die stabiliteit van die glas getoon nie, aangesien dit die vog slegs op die oppervlak adsorbeer het. Toenemende waterinhoud (tot en met 50 %) het geen plastiserende effek op amorfe asitromisien gehad nie. Die glas het ongeveer 339 % beter wateroplosbaarheid in
teenstelling met die dihidraat van asitromisien aangetoon. Dit was voorts ook 39 % meer oplosbaar in fosfaatbuffer (pH 6.8).

Die dissolusie profiel van die bereide asitromisien (amorfe vorm) tablette in water was baie belowend, weens die verhoogde oplosbaarheid van hierdie vorm. Hoër asitromisien konsentrasies is vinniger verkry in vergelyking met die tablette wat asitromisien dihidraat-bevat het. Die deurlaatbaarheid van amorfe asitromisien oor die intestinale membraan by hoë pH-waardes (6.8 en 7.2) was beduidend beter as die van die dihidraat. Die pH-afhanklikheid van die deurlaatbaarheid van die asitromisien glas oor die intestinale weefsel is hierdeur beklemtoon.

Die verbeterde wateroplosbaarheid van amorfe asitromisien, tesame met die vinniger dissolusie-tempo, die stabilitéit en verhoogde deurlaatbaarheid mag uiteindelik tot verhoogde biobeskikbaarheid na orale toediening lei.

Hierdie navorsingsuitkomstes het dus die behoefte laat ontstaan om die impak van die toediening van laer dosisse te ondersoek, ten einde te bepaal of dieselfde antimikrobiese effektiwiteit moontlik bereik kan word, weens die handhawing van identiese weefselkonsentrasies ten spyte van hierdie laer dosisse.

Sleutelwoorde: Makrolied; Asitromisien; Amorf; Oplosbaarheid; Stabiliteit; Dissolusie; Deurlaatbaarheid.
HPLC method development

- Developing a robust HPLC method for identification and quantification of azithromycin

Polymorphism of azithromycin

- Screening of different solid state forms produced through recrystallisation
- Preparation of anhydrous azithromycin
- Characterisation of anhydrous azithromycin

Amorphism of azithromycin

- Preparation of amorphous azithromycin glass
- Characterisation of azithromycin glass
- Solubility and stability of azithromycin glass

Product development

- Formulating azithromycin glass in a tablet
- Dissolution profiles of prepared tablets in various aqueous media
- Stability of azithromycin glass in tablet formulation

Permeability of azithromycin glass at various pH values