

Polimery w Medycynie

Polymers in Medicine

BIENNIAL ISSN: 0370-0747 e-ISSN: 2451-2699

www.polimery.umed.wroc.pl

2017, Vol. 47, No. 1 (January–June)

Ministry of Science and Higher Education – 9 pts.
Index Copernicus (ICV) – 109.18 pts.



WROCLAW
MEDICAL UNIVERSITY

Polimery w Medycynie

Polymers in Medicine

ISSN 0370-0747 (PRINT)

ISSN 2451-2699 (ONLINE)

www.polimery.umed.wroc.pl

BIANNUAL
2017, Vol. 47, No. 1
(January–June)

Polymers in Medicine is an independent, multidisciplinary forum to exchange scientific and clinical information, which publishes original papers (technical, analytical, experimental, clinical), preliminary reports and reviews regarding the use of polymers (natural and synthetic) and biomaterials in different specialties of medicine (biochemistry, clinical medicine, pharmacology, dentistry, implantology), biotechnology and veterinary science.

Address of Editorial Office

Marcinkowskiego 2–6
50-368 Wrocław, Poland
Tel.: +48 71 784 11 33
E-mail: polimery@umed.wroc.pl

Publisher

Wrocław Medical University
Wybrzeże L. Pasteura 1
50-367 Wrocław, Poland

© Copyright by Wrocław Medical University,
Wrocław 2017

Online edition is the original version of the journal

Editor-in-Chief

Magdalena Krajewska

Vice-Editor-in-Chief

Jerzy Gosk

Editorial Board

Rajmund Adamiec
Beata Dejak
Bożena Karolewicz
Witold Musiał

Secretary

Mariusz Kuształ

Michał Nachajski
Tadeusz Orłowski
Lidia Usnarska-Zubkiewicz
Włodzimierz Więckiewicz

Technical Editorship

Adam Barg, Paulina Kunicka,
Aleksandra Raczkowska

English Language Copy Editors

Sherill Howard-Pociecha, Jason Schock,
Marcin Tereszewski

Statistical Editors

Dorota Diakowska, Leszek Noga

International Advisory Board

Jenifer B. Dressman (Germany)
Mirosława El Fray (Poland)
Mukesh G. Gohel (India)
Vipin B. Gupta (India)
Anthony J. Hickey (USA)
Jacek Kaczmarczyk (Poland)
Agnieszka Noszczyk-Nowak (Poland)
Paweł Reichert (Poland)
Maciej Urban (Poland)
Timothy S. Wiedmann (USA)
Katarzyna Winnicka (Poland)
Waldemar Wysokiński (USA)
Samuel Yalkowsky (USA)

Editorial Policy

During the review process, the Editorial Board conforms to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication" approved by the International Committee of Medical Journal Editors (<http://www.icmje.org/>). Experimental studies must include a statement that the experimental protocol and informed consent procedure were in compliance with the Helsinki Convention and were approved by the ethics committee.

Subscription conditions

Requests for subscriptions should be directed to the Wrocław Medical University Press:

Wrocław Medical University Press

Polimery w Medycynie

Marcinkowskiego 2–6, 50–368 Wrocław, Poland

E-mail: zofia.gacek@umed.wroc.pl

For more information visit the following page: <http://www.polimery.umed.wroc.pl>

Indexed in: OCLC, WorldCat, PBL, EBSCO, MEDLINE, Index Copernicus

This publication has been co-financed by the Ministry of Science and Higher Education

Typographic design: Monika Kołęda, Piotr Gil

Cover: Monika Kołęda

DTP: Wrocław Medical University Press

Printing and binding: Wrocławska Drukarnia Naukowa PAN

Circulation: 90 copies

Contents

Original papers

- 5 Surya Narayan Ratha Adhikari, Satyabrata Panda
Buccal patches of atenolol formulated using fenugreek (*Trigonella foenum-graecum* L.) seed mucilage
- 13 John Oluwasogo Ayorinde, Michael Ayodele Odeniyi, Arvind K. Bansal
Evaluation of two novel plant gums for bioadhesive microsphere and sustained-release formulations of metformin hydrochloride
- 25 Tolulope Omolola Ajala, Hope Idemudia Olaiya, Oluwatoyin Adepeju Odeku
Film forming properties of *Cissus pulpunea* (Guill and Perr) and *Irvingia gabonensis* (O'Rorke) gums
- 35 Michael Ayodele Odeniyi, Babatunde Mukhtar Oyedokun, Oluyemisi Adebawale Bamiro
Native and microwave-modified *Terminalia mantaly* gums as sustained-release and bioadhesive excipients in naproxen matrix tablet formulations

Reviews

- 43 Michał Bąk, Olga N. Gutkowska, Ewa Wagner, Jerzy Gosk
The role of chitin and chitosan in peripheral nerve reconstruction
- 49 Marek Stępniewski, Jacek Martynkiewicz, Jerzy Gosk
Chitosan and its composites: Properties for use in bone substitution
- 55 Wojciech Satora, Aleksandra Królikowska, Andrzej Czamara, Paweł Reichert
Synthetic grafts in the treatment of ruptured anterior cruciate ligament of the knee joint

Buccal patches of atenolol formulated using fenugreek (*Trigonella foenum-graecum* L.) seed mucilage

Surya Narayan Ratha Adhikari^{A–E}, Satyabrata Panda^{C,E,F}

Department of Pharmaceutics, Seemanta Institute of Pharmaceutical Sciences, Mayurbhanj, Odisha, India

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article

Polymers in Medicine, ISSN 0370-0747 (print), ISSN 2451-2699 (online)

Polim Med. 2017;47(1):5–11

Address for correspondence

Surya Narayan Ratha Adhikari
E-mail: snarayan.rath@rediffmail.com

Funding sources

none declared

Conflict of interest

none declared

Acknowledgements

The authors are grateful to Dr. Amit Kumar Nayak, Department of Pharmaceutics, Seemanta Institute of Pharmaceutical Sciences, Mayurbhanj, Odisha, India.

Received on February 7, 2016

Revised on February 24, 2016

Accepted on April 18, 2017

Abstract

Background. The use of mucoadhesive natural polymers in designing mucoadhesive patch systems has received much attention.

Objectives. The study involved the development and evaluation of buccal patches of atenolol using fenugreek (*Trigonella foenum-graecum* L.) seed mucilage with hydroxypropyl methyl cellulose (HPMC K4M) and a backing membrane (ethyl cellulose 5% w/v).

Material and methods. These atenolol-releasing buccal patches were prepared using a solvent casting technique. The buccal patches prepared were evaluated for average weight, thickness, drug content, folding endurance and moisture content. Ex vivo mucoadhesive strength, force of adhesion and bonding strength were determined using porcine buccal mucosa. The mucosal permeation of atenolol through the porcine buccal mucosa was carried out using a Franz diffusion cell in phosphate buffer saline, pH 6.8. These buccal patches were also characterized by SEM and FTIR spectroscopy.

Results. The average weight, thickness, drug content, folding endurance and moisture content of these atenolol-releasing buccal patches were found satisfactory for all the patches. Amongst all, the F-4 buccal patch showed maximum mucoadhesive strength (31.12 ± 1.86 g), force of adhesion (30.53×10^{-2} N) and bond strength (1748.89 N/m²). Ex vivo atenolol permeation from the buccal patches showed drug permeation across the excised porcine buccal mucosa over 12 h. The F-4 buccal patch showed maximum permeation flux (29.12 µg/cm²/h).

Conclusions. The developed atenolol-releasing buccal patches can be beneficial over the conventional drug delivery systems to decrease the dosing frequency and enhance patient compliance.

Key words: fenugreek seed mucilage, HPMC, mucoadhesion, buccal patches, atenolol

DOI

10.17219/pim/70498

Copyright

© 2017 by Wrocław Medical University

This is an article distributed under the terms of the
Creative Commons Attribution Non-Commercial License
(<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

During last few decades, a lot of research work has been done in buccal drug delivery systems.^{1–3} Delivery of drugs via the buccal mucosa to the systemic circulation is defined as buccal drug delivery.⁴ Though less permeable than the sublingual area, the buccal mucosa is well vascularized and drugs can be rapidly absorbed into the systemic circulation underneath the oral mucosa.⁵ The mucosa of the buccal area has a large, smooth and relatively immobile surface, which provides a larger contact surface. The large contact surface of the buccal mucosa contributes to rapid and extensive drug absorption.⁶ In recent years, buccal drug delivery has proven particularly useful and offers several advantages over other drug deliveries including: bypass of the gastrointestinal tract and hepatic portal systems, increasing the bioavailability of orally administered drugs that otherwise undergo hepatic first-pass metabolism, improved patient compliance due to the elimination of associated pain with injections, administration of drugs to unconscious patients, sustained drug delivery, increased ease of drug administrations and ready termination of delivery by detaching the dosage form.⁷ In current years, several buccal patches of different drugs have been researched by various research groups.^{8–11} Buccal patches are modified-release dosage forms composed of a thin matrix composed of one or more polymers, drugs and other excipients. In the previous literature, a few attempts have been undertaken to design atenolol-releasing buccal films and patches.^{9,12–15} However, in the previous literature, no attempt has been undertaken to formulate atenolol patches using plant-derived natural mucoadhesive polymers along with hydroxylpropyl methyl cellulose (HPMC). HPMC is a release-retardant polymer. Therefore, it was thought to provide delayed release of atenolol from these buccal patches for a longer period.

The present research work involves the formulation and development of atenolol (an anti-hypertensive drug) releasing buccal patches made of fenugreek (*Trigonella foenum-graecum* L.) seed mucilage (a plant-derived mucoadhesive biopolymer) and HPMC so that the various preparations obtained sustained a prolonged drug release profile with suitable mucoadhesive properties. Fenugreek seed mucilage is a galactomannan and stable polymer.¹⁶ It consists of a (1→4) β -D-mannan backbone to which single α -D-galactopyranosyl groups are attached at the O-6 position of D-mannopyranosyl residues with D-galactose and D-mannose ratio, 1 : 1 or 1 : 1.2.^{16,17} The present study

focuses on the formulation and evaluation of atenolol-releasing buccal patches made of a mucoadhesive polymeric layer of fenugreek seed mucilage-HPMC and a drug-free backing membrane consisting of 1% w/v ethyl cellulose. The 1% w/v ethyl cellulose was used as backing layer.

Material and methods

Material

Atenolol was obtained from M/S. P.D.I.L, India. HPMC K4M and ethyl cellulose were obtained from Matrix Laboratories, India. Glycerin was purchased from Loba Chemie Pvt. Ltd., India. Sodium saccharin was purchased from Reidel India Chemicals, India. Fenugreek (*Trigonella foenum-graecum* L.) seed mucilage was isolated from the raw fenugreek seeds. The procedure of fenugreek seed mucilage extraction has been described in previously published literature by Nayak et al.^{16,17} The chemicals used were of analytical grade and double-distilled water was used throughout.

Preparation of buccal patches of atenolol

The buccal patches consisting of mucoadhesive polymeric layers of fenugreek seed mucilage-HPMC containing atenolol (50 mg) and sodium saccharin (0.1% w/v) were prepared by a solvent casting technique. The mixture solutions of atenolol, mucoadhesive polymers (fenugreek seed mucilage and HPMC), and sodium saccharin were well mixed using a magnetic stirrer (Remi Motors, India) at 100 rpm for 15 min and then, homogenized using a homogenizer stirrer (Remi Motors, India) for 15 min. Glycerin was used as a plasticizer within the mixture solution at a concentration of 15% w/w of dry weight of the polymers. The solutions were then sonicated again for 30 min to remove air bubbles. The sonicated solutions were poured in petri dishes of 54 cm² area and were dried at 50°C for 24 h. The drug-free backing layer was prepared onto the drug containing mucoadhesive polymeric layers by using 1% w/v ethyl cellulose solution in ethyl alcohol by a solvent casting technique and the prepared bilayered patches were dried at 50°C for 5 h. Then, the dried atenolol patches were obtained from the petri dishes, cut into circular pieces of 1 cm² and appropriately preserved. Table 1 shows the composition of different buccal patches containing atenolol.

Measurement of average weight and thickness

The average weights of all the patches were calculated including the measurement of their thickness at 6 different points using thickness gauge (Mitutoyo, Japan). For each formulation, 3 randomly-selected patches were used.⁹

Table 1. Composition of different buccal patches containing atenolol

Formula	F-1	F-2	F-3	F-4
Atenolol (mg)	50	50	50	50
HPMC K4M (mg)	500	400	300	200
Fenugreek seed mucilage (mg)	500	600	700	800
Glycerin (% w/w)	15	15	15	15
Sodium saccharin (% w/v)	0.1	0.1	0.1	0.1
Distilled water (mL)	40	40	40	40

Determination of drug content

The drug content uniformity in the formulated buccal patches were determined by weighing 6 patches (1 cm²) dissolved in 100 mL isotonic phosphate buffer (pH 6.8) and shaken vigorously for 24 h at room temperature. Then the resulting solution was filtered, diluted and analyzed for DS content spectrophotometrically using a UV–VIS spectrophotometer (UV-1700 Double beam spectrophotometer, SHIMADZU Corporation, Japan) at 274 nm against a blank. The drug content was estimated from the calibration curve, which was constructed between 1 and 5 µg/mL concentration ranges. The method was validated for linearity, accuracy, and precision. The regression equation for the calibration curve was $Y = 0.048 X + 0.002$, $R^2 = 0.9990$.

Measurement of folding endurance

The folding endure without breakage of the patches was determined by repeatedly folding one patch at the same place till it broke or folded up to 300 times at the same place without break. The number of times the buccal patches could be folded at the same place without breaking or cracking gave the value of folding endurance.¹⁸

Determination of moisture content

The buccal patches were weighed accurately and kept in desiccators containing anhydrous calcium chloride. After 3 days, the patches were taken out and weighed.¹⁹ The moisture content (%) was determined by calculating moisture loss (%) using the formula:

$$\frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100 \quad (1).$$

Ex vivo studies

Preparation of goat buccal mucosa

The goat buccal mucosa excised from a goat cheek pouch was obtained within 2 h of its death from the slaughter house and kept immediately in phosphate buffer solution. The buccal mucosa was isolated from the full thickness of the tissue and finally washed with phosphate buffer saline, pH 6.8.

Ex vivo mucoadhesion study

The mucoadhesive parameters (mucoadhesive strength, force of adhesion and bonding strength) of all the fabricated buccal patches were measured ex vivo ($n = 3$) by a modified physical balance using excised porcine buccal mucosa as described by Gupta et al.²⁰ A piece of excised

porcine buccal mucosa was tied to the open mouth of a glass vial filled completely with phosphate buffer saline, pH 6.8. The glass vial was tightly fitted in the center of a beaker filled with phosphate buffer saline (pH 6.8; temperature, $37 \pm 1^\circ\text{C}$). The patches were stuck to the lower side of the rubber stopper with glue. The mass (in g) required to detach the patches from the mucosal surface gave the measure of mucoadhesive strength (shear stress). The following parameters were calculated from mucoadhesive strength:¹⁰

$$\text{force of adhesion (N)} = \frac{\text{mucoadhesive strength} \times 9.81}{1000}$$

$$\text{bonding strength (N/m}^2\text{)} = \frac{\text{force of adhesion}}{\text{surface area of mucosal surface}} \quad (2).$$

Ex vivo permeability study

The formulated buccal patches that were evaluated for the permeation of atenolol through the porcine buccal mucosa were carried out using a Franz diffusion cell. The effective diffusion area was 1.74 cm². The receptor compartment (40 mL) was filled with phosphate buffer saline, pH 6.8, and its temperature was maintained at $37 \pm 1^\circ\text{C}$. The buccal cavity environment was stimulated by applying a magnetic stirrer (100 rpm). The patch was applied under occlusion on the buccal mucosal surface fitted between the donor and receptor compartments of the diffusion cell. Five milliliters of the sample from the receptor medium was withdrawn at predetermined time intervals and an equal volume of pre-warmed phosphate buffer saline pH 6.8 was replaced immediately. The amount of atenolol released into the receptor compartment was quantified by using a UV–VIS spectrophotometer (UV-1700 double beam spectrophotometer, SHIMADZU Corporation, Japan) at 274 nm against a blank.

Ex vivo permeation data analysis

Permeation flux: The amount of atenolol from the various buccal patches permeated through the excised porcine buccal mucosa was plotted against the function of time. The slope and intercept of the linear portion of the plots were derived by regression. The permeation fluxes for each patch were calculated as the slope divided by the mucosal surface area.¹⁴

$$J_{ss} = (dQ/dt)_{ss} \cdot 1/A \quad (3),$$

where J_{ss} is the steady-state permeation flux (µg/cm²/h), A is the area of buccal mucosa (cm²) through which drug permeation takes place, and $(dQ/dt)_{ss}$ is the amount of drug passing through the buccal mucosa per unit time at a steady state (µg/h).

Table 2. Average weight, thickness, drug content, folding endurance and moisture content of atenolol-containing buccal patches

Formula	F-1	F-2	F-3	F-4
Average weight (g) ^a	2.07 ±0.08	2.17 ±0.09	2.08 ±0.07	2.09 ±0.07
Thickness (mm) ^b	0.58 ±0.06	0.60 ±0.04	0.57 ±0.06	0.58 ±0.05
Drug content (%) ^b	99.48 ±2.78	99.02 ±2.57	98.37 ±2.68	98.66 ±2.43
Folding endurance	88	83	82	80
Moisture content (%) ^b	1.21 ±0.09	1.22 ±0.07	1.36 ±0.09	1.50 ±0.12

^a mean ± standard deviation, n = 6.^b mean ± standard deviation, n = 3.

Permeation kinetics: The data of ex vivo atenolol permeation from the various buccal patches through excised porcine buccal mucosa were evaluated kinetically using various mathematical models like zero order, first order, Higuchi, and Korsmeyer-Peppas model equations.²¹

Zero order model: $F = K_0 t$, where F represents the fraction of the drug permeated in time t and K_0 is the zero order rate constant.

First order model: $\ln(1 - F) = -K_1 t$, where F represents the fraction of the drug permeated in time t and K_1 is the first-order rate constant.

Higuchi model: $F = K_H t^{1/2}$, where F represents the fraction of the drug permeated in time t and K_H is the Higuchi rate constant.

Korsmeyer-Peppas model: $F = K_p t^n$, where F represents the fraction of the drug permeated in time “t” and K_p is the Korsmeyer-Peppas rate constant, and “n” is the diffusion exponent.

Again, the Korsmeyer-Peppas model was employed in the ex vivo atenolol permeation behavior analysis of these formulations to find out permeation mechanisms: Fickian (non-steady) when $n \leq 0.5$, case-II transport (zero order) when $n \geq 1$, and non-Fickian (anomalous) when the value of n is in between 0.5 and 1.²²

Analysis of surface morphology

The surface morphology of these patches was examined by scanning electron microscopy (SEM). The dried patches were coated with gold sputter and then observed under scanning electron microscope (JEOL, JSM 840, Japan).

Analysis of drug-polymer compatibility

The drug-polymer compatibility of the prepared patches was analyzed by Fourier transform-infrared (FTIR) spectroscopy. Pure drug (atenolol) and atenolol-containing buccal patches were scanned over a wave number range of 3600 to 600 cm^{-1} at a resolution of 4 cm^{-1} using a FTIR spectrophotometer (BRUKER). The system was operated in transmission mode.

Statistical analysis

All data was analyzed with simple statistics. The simple statistical analysis was conducted using MedCalc software v 11.6.1.0.

Results and discussion

The main aim of the research work was to formulate and evaluate atenolol-(an anti-hypertensive drug) releasing buccal patches containing the drug in a mucoadhesive polymeric layer of fenugreek seed mucilage-HPMC and a drug-free backing membrane composed of 1% w/v ethyl cellulose using the solvent casting technique.

Average weight and thickness

The average weights and thicknesses of these atenolol-containing buccal patches as a whole (54 cm^2) were measured within the range, 2.07 ±0.08 to 2.17 ±0.09 g and 0.57 ±0.06 to 0.60 ±0.04 mm, respectively (Table 2).

Drug content

The uniformity of drug contents in 1 cm^2 of each buccal patch were determined. The drug contents in all these buccal patches varied between the range 98.37 ±2.68 and 99.48 ±2.78% (Table 2). This shows that the drug dispersed uniformly throughout the drug-containing polymeric layer.

Folding endurance

The folding endurances of these formulated buccal patches were measured manually. The highest folding endurance was observed in the case of F-1 (18) and the lowest in the case of F-4 (10) (Table 2). It was observed that the folding endurances of the patches were found to be decreased with the decrease of fenugreek seed mucilage within the formula of these patches. The folding endurance study signifies flexibility of the designed buccal patches.

Moisture content

The percentages of moisture content (%) of all these atenolol-containing buccal patches were measured within the range 1.21 ±0.09 to 1.50 ±0.12% (Table 2). The low moisture content protects it from microbial contamination, reduces the bulkiness of the formulated patches and gives it stability from brittleness.¹⁴

Table 3. Mucoadhesive parameters (ex vivo mucoadhesive strength, force of adhesion and bonding strength) of atenolol-containing buccal patches

Formulations	F-1	F-2	F-3	F-4
Mucoadhesive strength in (g) ^a	21.48 ±1.36	23.12 ±1.27	26.82 ±1.44	31.12 ±1.86
Force of adhesion (N) ^a	21.07 × 10 ⁻²	22.68 × 10 ⁻²	26.31 × 10 ⁻²	30.53 × 10 ⁻²
Bonding strength (N/m ²) ^a	1211.03	1303.49	1512.09	1748.89

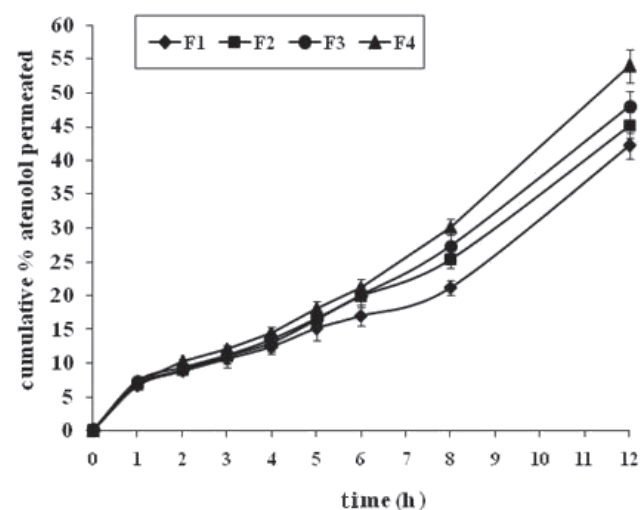
^a mean ± standard deviation, n = 3.

Ex vivo mucoadhesion

The mucoadhesion of buccal patches may be defined as the adhesion between the buccal patches and buccal mucosa. The strength of the mucoadhesion is affected by various factors such as the biological membrane used in the study, molecular mass, and the swelling rate of the polymers present in the formulation.¹³ In this investigation, freshly excised porcine buccal mucosa was used as the biological membrane. Various mucoadhesive parameters like ex vivo mucoadhesive strength (in gram), force of adhesion (N) and bonding strength (N/m²) were measured by a modified physical balance using excised goat buccal mucosa as described by Gupta et al.²⁰ The results of the ex vivo mucoadhesion study of atenolol-containing buccal patches using excised porcine buccal mucosa are presented in Table 3. Among all these formulated patches, the F-4 atenolol-containing buccal patch showed maximum mucoadhesive strength (31.12 ±1.86 g), force of adhesion (30.53 × 10⁻² N), and bond strength (1748.89 N/m²). It was observed that the mucoadhesion of these patches was found to be increased with the increment of fenugreek seed mucilage incorporation in the buccal patch formula as the mucoadhesive polymer. The ex vivo mucoadhesive strengths, forces of adhesion and bonding strengths of these newly-formulated atenolol-containing buccal patches were found satisfactory for mucoadhesion in the oral cavity with the buccal mucosal surface.

Ex vivo permeation

The ex vivo permeation of atenolol from the various buccal patches showed that the drug easily permeates through

**Fig. 1.** Ex vivo atenolol permeation from the various atenolol-containing buccal patches

the excised goat buccal mucosa during a period of 12 h and is shown in Fig. 1. It was further found that the maximum ex vivo drug permeation was 50.36 ±2.43% over a period of 12 hours in the case of the F-4 atenolol-containing buccal patch, while the minimum ex vivo drug permeation was found to be 42.17 ±1.88% over a period of in the case of the F-1 atenolol-containing buccal patch. The permeation fluxes (J, µg/cm²/h) were calculated for each buccal patch and are presented in Table 4. The permeation flux results showed the maximum permeation flux (29.12 µg/cm²/h) for the F-4 atenolol-containing buccal patch, compared to that of the others.

To discover the permeation of atenolol from different buccal formulations, it is necessary to fit it into a suitable mathematical model. The ex vivo atenolol permeation data from buccal patches across the excised porcine buccal mucosa were evaluated kinetically using various mathematical models like zero order, first order, Higuchi, and Korsmeyer-Peppas model equations.²¹ The results of the curve fitting of the ex vivo atenolol permeation data into these above-mentioned mathematical models indicate the atenolol permeation behavior from these formulated buccal patches of atenolol across the excised porcine buccal mucosa (Table 5). The ex vivo atenolol permeations were found to follow the first order model ($R^2 = 0.9858$ to 0.9943) over a period of 12 h of permeation across the excised porcine buccal mucosa. The values determined for the diffusion exponent (n) of the prepared atenolol-containing buccal patches ranged between 0.69 and 0.76 (Table 5) indicating a non-Fickian (anomalous) diffusion mechanism of drug permeation. The anomalous diffusion mechanism describes both diffusion-controlled, and swelling-controlled drug permeation.²⁴

Table 4. Permeation flux (J, µg/cm²/h) results for the various atenolol-containing buccal patches

Formulations	Permeation flux (J, µg/cm ² /h)
F-1	24.87
F-2	25.03
F-3	27.28
F-4	29.12

Table 5. Results of the curve fitting of the ex vivo permeation data of the various atenolol-containing buccal patches

Formulation code	F1	F2	F3	F4
Zero order model	0.9375	0.9637	0.9638	0.9552
First order model	0.9881	0.9858	0.9913	0.9943
Higuchi model	0.6457	0.6385	0.6361	0.6099
Korsmeyer-Peppas model	0.9134	0.9308	0.9161	0.9072
n (diffusion exponent)	0.69	0.72	0.74	0.76

Surface morphology

An SEM microphotograph of the F-4 atenolol-containing buccal patch is shown in Fig. 2. It shows a nearly smooth surface with the presence of very few drug particles. This indicated good lamination of the polymers within the patch, where the drug particles were homogeneously dispersed throughout the patch matrix. The presence of drug particles on the surface of the patch matrix might be a result of their migration along with water to the patch surface during drying.

Drug-polymer compatibility

The drug-polymer compatibility of the prepared buccal patches was analyzed by FTIR spectroscopy. The FTIR spectra of fenugreek seed mucilage, HPMC K4M, ethyl cellulose, the F-4 atenolol-containing buccal patch and the pure drug (atenolol) are presented in Fig. 3. The FTIR spectrum of pure atenolol showed various characteristic peaks of atenolol like at 3305 and 1416 cm^{-1} due to -O-H, at 1337 cm^{-1} due to -CH₃, and at 1036 and 1242 cm^{-1} due to -N-C, as expected.²⁵ All these characteristic peaks of pure atenolol appeared in the spectra of the F-4 atenolol-containing buccal patch without or with very minute shifting, indicating that there was an absence of any chemical interaction between the drug (here atenolol) and the excipients used. The intensity of the peaks of pure atenolol was diminished due to the molecular dispersion of the atenolol in the polymer matrix of the buccal patch.

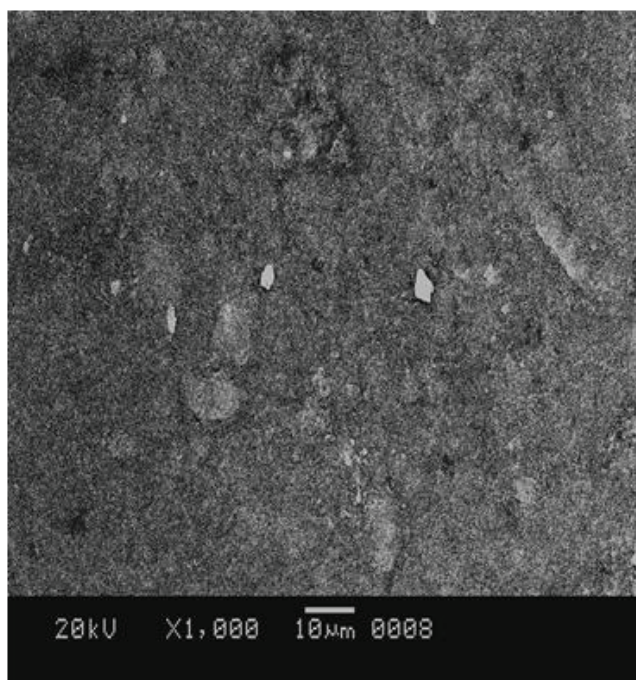


Fig. 2. SEM microphotograph of the F-4 atenolol-containing buccal patch

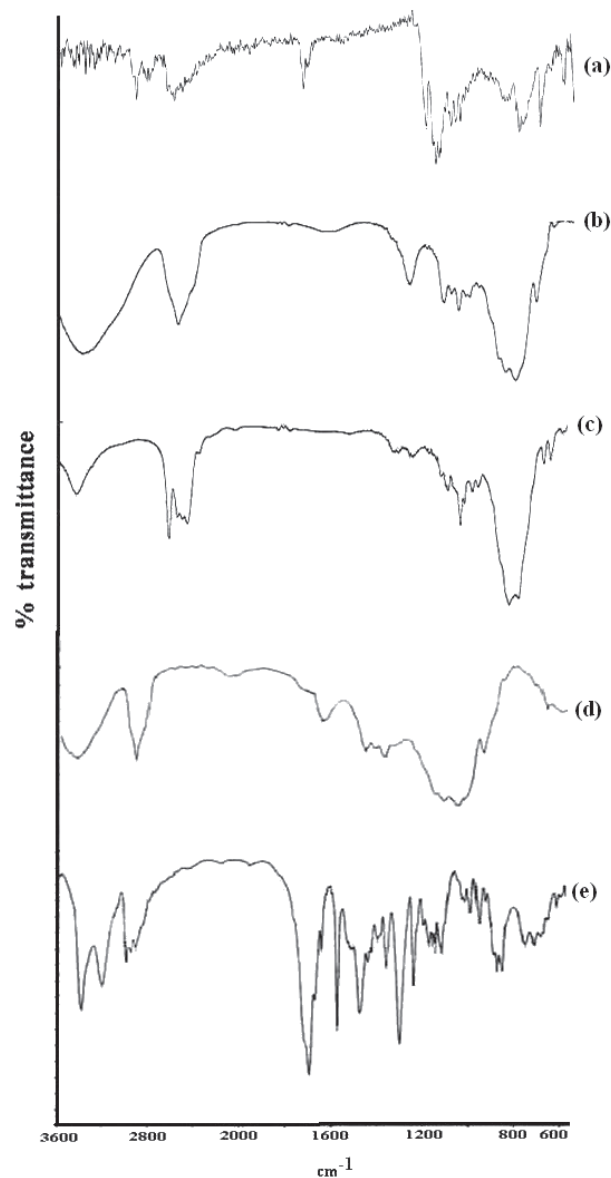


Fig. 3. The FTIR spectra of fenugreek seed mucilage (a), HPMC K4M (b), ethyl cellulose (c), the F-4 atenolol-containing buccal patch (d) and the pure drug (atenolol)

Conclusion

From the present work, it can be concluded that in such mucoadhesive buccal patches of atenolol made of a mucoadhesive polymeric layer of fenugreek seed mucilage-HPMC and a drug-free backing membrane composed of 1% w/v ethyl cellulose using a solvent casting technique, 1% w/v ethyl cellulose was found suitable to provide a sustained buccal delivery of atenolol for prolonged periods in the management of hypertension, which can be a good way to bypass the extensive hepatic first-pass metabolism. In the future, the in vivo ability of these newly-developed atenolol-releasing buccal patches will be investigated.

References

1. Hao H, Heng PWS. Buccal drug delivery systems. *Drug Dev Ind Pharm*. 2003;29:821–832.
2. Avachat AM, Gujar KN. Development and evaluation of tamarind seed xyloglucan-based mucoadhesive buccal films of rizatriptan benzoate. *Carbohydr Polym*. 2013;91:537–542.
3. Koland M, Charyulu RN, Prabhu P. Mucoadhesive films of losartan potassium for buccal delivery: Design and characterization. *Indian J Pharm Educ Res*. 2010;44:315–323.
4. Boylan JC. Drug delivery buccal route. In: J. Swarbrick (ed.), *Encyclopedia of Pharmaceutical Technology*. Suppl. 3, Marcel Dekker Inc 2001, 800–811.
5. De Vries ME, Bodde HE, Verhoef JC, Junginger HE. Developments in buccal drug delivery. *Crit Rev Ther Drug Carrier Syst*. 1991;8:271–303.
6. Shojaei AH. Buccal mucosa as a route for systemic drug delivery. *Review. J Pharm Pharmaceut Sci*. 1998;1:15–30.
7. Smart JD. Buccal drug delivery. *Exp Opin Drug Deliv*. 2005;2:507–517.
8. Shiledar RR, Tagalpallewar AA, Kokare CR. Formulation and in vitro evaluation of xanthan gum-based bilayered mucoadhesive buccal patches of zolmitriptan. *Carbohydr Polym*. 2014;101:1234–1242.
9. Verma N, Wahi AK, Verma A, Chattopadhyay P. Evaluation of mucoadhesive buccal patches for delivery of atenolol. *J Pure Appl Microbiol*. 2007;1:115–118.
10. Patel VM, Prajapati BG, Patel MM. Design and characterization of chitosan- containing mucoadhesive buccal patches of propranolol hydrochloride. *Act Pharm*. 2007;57:61–72.
11. Pongjanyakul T, Suksri H. Alginate-magnesium aluminum silicate films for buccal delivery of nicotine. *Colloids Surf B Biointerf*. 2009;74:103–113.
12. Jug M, Bećirević-Laćan M, Bengez S. Novel cyclodextrin-based film formulation intended for buccal delivery of atenolol. *Drug Dev Ind Pharm*. 2009;35:796–807.
13. Krishnarth N, Verma N, Sharma N. Formulation & evaluation of mucoadhesive buccal patches for delivery of atenolol. *Int J Pharm*. 2014;4:166–170.
14. Ratha Adhikari SN, Nayak BS, Nayak AK, Mohanty B. Formulation and evaluation of buccal patches for delivery of atenolol. *AAPS PharmSciTech*. 2010;11:1038–1044.
15. Satishbabu BK, Srinivasan BP. Preparation and evaluation of buccal adhesive films of atenolol. *Indian J Pharm Sci*. 2008;70:175–179.
16. Nayak AK, Pal D, Pradhan J, Ghorai T. The potential of *Trigonella foenum-graecum* L. seed mucilage as suspending agent. *Indian J Pharm Educ Res*. 2012;46:312–317.
17. Nayak AK, Pal D, Pradhan J, Hasnain MS. Fenugreek seed mucilage-alginate mucoadhesive beads of metformin HCl: Design, optimization and evaluation. *Int J Biol Macromol*. 2013;54:144–154.
18. Nafee NA, Ahemed F, Borale A. Preparation and evaluation of mucoadhesive patches for delivery of cetylpyridinium chloride (CPC). *Acta Pharm*. 2003;2:199–212.
19. Attama AA, Akpa PA, Onugwa LE, Igwilo G. Novel buccoadhesive delivery system of hydrochlorothiazide formulated with ethyl cellulose-hydroxypropyl methylcellulose interpolymers complex. *Sci Res Essays*. 2008;3:343–347.
20. Gupta A, Garg S, Khar RK. Measurement of bioadhesion strength of muco-adhesive buccal tablet: Design of an in vitro assembly. *Indian Drugs*. 1992;30:152–155.
21. Malakar J, Sen SO, Nayak AK, Sen KK. Formulation, optimization and evaluation of transferosomal gel for transdermal insulin delivery. *Saudi Pharm J*. 2012;20:355–363.
22. Malakar J, Sen SO, Nayak AK, Sen KK. Development and evaluation of microemulsions for transdermal delivery of insulin. *ISRN Pharmaceutics*. 2011 (2011) Article ID 780150.
23. Park H, Robinson JR. Mechanism of bioadhesion of poly (acrylic acid) hydrogels. *Pharm Res*. 1987;4:457–464.
24. Das B, Nayak AK, Nanda U. Topical gels of lidocaine HCl using cashew gum and Carbopol 940: Preparation and in vitro skin permeation. *Int J Biol Macromol*. 2013;62:514–517.
25. Cozar O, Chis V, David L, Baies M. Experimental and density functional theory investigation of some biomedical compounds. *J Optoelectronics Adv Mater*. 2006;8:164–171.

Evaluation of two novel plant gums for bioadhesive microsphere and sustained-release formulations of metformin hydrochloride

John Oluwasogo Ayorinde^{1,A–E}, Michael Ayodele Odeniyi^{1,C,E}, Arvind K. Bansal^{2,E,F}

¹ Department of Pharmaceutics and Industrial Pharmacy, University of Ibadan, Ibadan, Nigeria

² Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research, S.A.S. Nagar, India

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article

Polymers in Medicine, ISSN 0370-0747 (print), ISSN 2451-2699 (online)

Polim Med. 2017;47(1):13–23

Address for correspondence

John O. Ayorinde
E-mail: shogo205@yahoo.com

Funding sources

none declared

Conflict of interest

none declared

Received on October 12, 2016

Revised on March 14, 2017

Accepted on June 14, 2017

Abstract

Background. The biological half life of metformin requires multiple doses which are associated with poor patient compliance. This justifies the need for a dosage form with reduced dosing frequency.

Objectives. Gums from *Enterolobium cyclocarpum* and *Cedrela odorata* trees were evaluated in formulating bioadhesive microspheres containing metformin hydrochloride, for sustained drug release. Hydroxylpropyl-methyl cellulose (HPMC) was the standard.

Material and methods. Microspheres were produced from formulations of API and either cedrel gum (FC), enterolobium gum (FE) or HPMC (FH), using a W/O solvent extraction technique. The microspheres were characterized using a particle size analyzer, scanning electron microscopy (SEM), differential scanning calorimetry (DSC), powder X-ray diffractometer (PXRD), drug entrapment, in vitro release and mucoadhesion studies. The data was analyzed using ANOVA and t-test at $p = 0.05$.

Results. FT-IR spectroscopy indicated no alteration in the functional groups of metformin. A yield of 92–98% microspheres was obtained from all the formulations which had a particle size range of 72–84 μm . SEM revealed cylindrical to near-spherical particles with rough surfaces. The drug release profile showed a burst over the first 30 min followed by a steady release for about 5 h and a slow release for 5 days. Formulations containing the gums sustained the release of API for almost the same time as HPMC formulations; the ranking order was $\text{FE} > \text{FH} > \text{FC}$ ($p > 0.05$). All the formulations exhibited good concentration-dependent mucoadhesive properties.

Conclusions. The gums were suitable for formulation of mucoadhesive microspheres for sustained release of metformin. The formulations showed good release properties in an alkaline pH.

Key words: microspheres, metformin, bioadhesion, enterolobium gum, cedrel gum

DOI

10.17219/pim/74776

Copyright

© 2017 by Wrocław Medical University

This is an article distributed under the terms of the
Creative Commons Attribution Non-Commercial License
(<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

Microspheres are small, spherical particles with a diameter in the micrometer range (1–1000 μm), obtainable from synthetic and natural materials, suitable for drug loading and release. Drug delivery systems that couple mucoadhesive properties to microspheres would be capable of enhancing intimate contact, better bioavailability, specific drug targeting and sustained release properties.

The potentially toxic effects of synthetic polymers is a major drawback in their use as drug carriers. Hence, the use of natural polymers that are biodegradable, cheap and easily accessible is becoming important in the design of drug delivery systems. Bioadhesion is a process by which macro-molecules stick to the mucosal surfaces in the body and remain there for a reasonable length of time. When these materials are loaded with active pharmaceutical ingredients (API), they enhance the release of the drug substance for either local or systemic absorption. A more specific term is mucoadhesion; adhesion of materials to surfaces of the body such as the nose and mouth that are covered by mucin molecules.¹ Mucoadhesion has been defined as the interfacial force interaction between polymeric materials and mucosal tissues.² Mucoadhesive agents are usually polymers and they contain hydrogen bonds, useful in wet formulations or in dry powders for drug delivery purposes. For mucoadhesion to occur, there must be close contact between the mucoadhesive agent and the mucus, followed by the formation of chemical bonds between the macro-molecules.

Plant gums are polysaccharides of natural origin. They are adhesive in nature and produced as exudates from the incised bark of trees and shrubs. Plant gums are generally hydrophilic with some being soluble in water while others produce mucilages by absorbing water. Gums have been found to be useful in the food, pharmaceutical, paper and textile industries.³

The *Cedrela odorata* tree, from which cedrela gum is obtained, is an important tree species in the family chinaberry, *Meliaceae*. It is commonly called Spanish cedar or Cuban cedar. It is a tree in the new world tropics appearing in the forests of moist and seasonally dry subtropical or tropical life zones. Its primary use is in storing household clothing. It contains aromatic and insect repelling resins and is often used in honey production. The polysaccharides from the gum contain galactose, arabinose and rhamnose as neutral sugars and uronic acids as residues. The cationic components of the ash are mainly calcium and magnesium. Cedrela gum has a non-Newtonian flow behavior characterized by lack of a low-shear limiting Newtonian viscosity plateau even at low shear rates. The average flow index value is low but infinite shear rate viscosity is high.⁴ Previous works showed cedrela gum to have mucoadhesive properties.⁵

Enterolobium gum, produced by the *Enterolobium cyclocarpus* tree family *Mimosoideae* of the *Leguminosae*, is commonly known as the guanacaste or elephant-ear tree. It is a species of flowering tree, native to Central

America and now widely distributed throughout the tropics where it is planted mainly as a roadside or garden tree.⁶ The tree is useful for its production of highly palatable and nutritious pods, containing sugary dry pulp. The pods are also used as food when cooked as a vegetable. Extracts from the bark have medicinal properties and have been used against colds and bronchitis.⁷ A structural study of the gum using chemical methods and NMR spectroscopy showed the structure is essentially a β -(1-3)-galactan, with the presence of α -L-arabinopyranose.⁸ The gum contains galactose, arabinose, rhamnose and glucuronic acid as the main monosaccharides, it has a high concentration of uronic acid and is highly viscous in nature.⁹ The use of enterolobium gum in pharmaceutical dosage forms has not yet been investigated.

The use of gums as polymers in pharmaceutical formulations has been reported; Akpabio et al.¹⁰ formulated and evaluated sustained release tablets produced from *Lesianthera africana* gum, Adedokun et al.¹¹ studied the compressional, mechanical and release properties of *Eucalyptus tereticornis* in paracetamol tablet formulations, Emeje et al.¹² worked on the formulation properties of *Cissus refescence* gum, while Odeniyi et al.² reported the release and mucoadhesive properties of diclofenac matrix tablets from natural and synthetic polymer blends. In the present study, we have investigated extracts from 2 native trees, whose use in drug formulation is not yet reported.

Hydroxy propyl methylcellulose (HPMC) was used as a standard polymer. It has a reversible thermal gelation property and forms hydrophilic matrices which mainly act by means of diffusion in controlling drug release. HPMC was used in this study due to its reported significant adhesive properties in tablet dosage forms.^{5,13}

Natural mucoadhesive substances considerably swell in water and form a gelatinous mass.¹⁴ This gelling and the mucoadhesive properties of cedrela gum have been reported.² The similarity in the polysaccharide composition of cedrela and enterolobium gums suggests similar mucoadhesive and gelling properties, hence the basis for comparison of these 2 natural polymers with HPMC as the standard. Formulating mucoadhesive microspheres from these natural polymers is expected to offer the possibility of intimate contact between a drug delivery system and mucous membranes, and sustained release of the loaded API.

Metformin is the first-line drug in the management of Type II diabetes. Metformin is believed to be the most widely orally used medication for diabetes and it is also used in polycystic ovary syndrome.¹⁵ Metformin has bioavailability of 50–60% under fasting conditions, it reaches peak plasma concentration within 1–3 h of administration of immediate-release and 4–8 h with extended-release formulations, with an average elimination half life of 6.2 h.¹⁶ The short elimination half life is a limitation which necessitates frequent administration, 2–3 times daily, leading to poor patient compliance and adherence.

The high release profile of metformin from pectin microspheres has been reported.¹⁷ Also, the dissolution rate of metformin hydrochloride in phosphate buffer (pH 6.8) was studied in different formulations and was found to be as high as between 96.27 and 97.93%.¹⁸

This paper evaluates the solid-state characteristics and physicochemical properties of 2 novel plant gums (enterolobium and cedrela gums) in the formulation of bioadhesive microspheres loaded with metformin hydrochloride with the target of reducing the dosing frequency of metformin through a sustained-release drug delivery system.

Material and methods

Material

The materials used in this work include enterolobium gum (ET), obtained from the *Enterolobium cyclocarpus* (Mimosoideae) tree, cedrela gum (CD), obtained from the *Cedrela odorata* (Meliaceae) tree, and hydroxy propyl methylcellulose (HPMC) from Colorcon Asa Limited India. Metformin hydrochloride, from Arbro Pharmaceuticals Limited, India was the model drug and the reagents were of AR grade.

Methods

Extraction of the gum

The cedrela and enterolobium gums were collected from *Cedrela odorata* and *Enterolobium cyclocarpus* trees, respectively, and authenticated at the Botany Department, University of Ibadan, Nigeria. The collected gum was purified using the established procedure and then hydrated by soaking in a chloroform/water mixture of 0.5/95.5% V/V for 5 days, while stirring from time to time.¹⁹ Unwanted materials were removed by straining the gum through a muslin cloth. The gum was precipitated from the solution by absolute ethanol, filtered and washed with diethyl ether and then dried in the oven at 40°C for 18 h to ensure complete removal of associated earth particle and toxic residues.^{20–22} The gum was milled in a domestic blender and sieved. Materials of particle size of < 200 µm were collected and used for all investigations.

Fourier Transform Infrared (FT-IR) spectroscopy

The possibility of interaction between the pure drug (metformin) and each of the polymers in the final formulations was established by recording their spectra on the FT-IR spectroscope (Model 2000 Perkin Elmer Spectroscopy, USA). Samples were prepared in KBr discs (1% w/w). A scanning range of 1000–4500 cm⁻¹ was used.

Preparation of samples for formulation

Material blends for microsphere formulations were made in their various proportions (Table 1), containing metformin and polymer in ratios 1 : 1, 1 : 2, 1 : 3 and 1 : 4. The component powders were mixed in a planetary mixer for 5 min to ensure homogeneity. Formulations were stored in air-tight containers.

Formulation of microspheres

Metformin microsphere beads were formulated by the W/O emulsion solvent evaporation technique.¹⁷ Different drug : polymer ratios were used (Table 1). The drug (500 mg) and the gum (500, 1000, 1500 or 2000 mg) were dispersed in water. The slurry formed was transferred into 200 mL of liquid paraffin and 0.5% Span 80 was added as the emulsifying agent. The system was emulsified by stirring in a 500 mL beaker at a temperature of 80°C and 200 rpm on a magnetic stirrer for 2.5 h. On evaporation of the aqueous phase, the oil was decanted to collect the microspheres formed. Filtration was carried out using no. 1 Whatman filter paper and the microspheres were washed repeatedly with n-hexane to remove the oil. The microspheres were dried in an oven at 60°C for 2 h and then stored in a desiccator over fused calcium chloride.

Evaluation of the microspheres

1. Drug content

A quantity of 100 mg microspheres was taken from each formulation and powdered using a mortar and pestle. The powder was suspended in methanolic water to form a 1 in 100 mL suspension. The suspension was agitated and then filtered through a 0.45 µm membrane filter. Metformin content was determined spectrophotometrically.

Table 1. Thermal behavior of polymers

Material	Onset temperature, T ₀ (°C)	Endset temperature, T _e (°C)	Peak, T _p (°C)	Enthalpy change, ΔH (J/g)
Cedrela gum	57.40	112.73	76.41	513.40
Enterolobium gum	50.06	139.98	77.47	705.31
HPMC	52.55	115.61	80.45	520.62
Metformin	221.45	225.65	222.45	450.55
FC 3	85.22	118.23	70.32	495.20
FE 3	62.45	122.55	72.12	500.52
FH 3	75.22	120.22	75.35	477.34

metrically at 233 nm using a regression equation from the standard calibration curve.

2. Percent microsphere yield

The yield was calculated as the weight of the microspheres recovered from each batch divided by the total weight of the API and polymer multiplied by 100.

3. Entrapment efficiency

A quantity (100 mg) of the drug-loaded microspheres was dispersed in 100 mL of methanolic water. The resultant dispersion was agitated and filtered through a 0.45 μm membrane filter. Drug content was determined spectrophotometrically at 233 nm, using a regression equation from a standard graph. Entrapment efficiency was calculated as follows:

$$EE = PC/TC \times 100 \quad (1),$$

where PC is the practical drug content and TC is the theoretical drug content. Determinations were done in triplicate.

4. Particle shape and morphology

The shape and surface topography of the microspheres were studied using a scanning electron microscope (Hitachi Japan, Model S3400N). Gold coating was used to make the samples electrically conductive.

5. Moisture content

The moisture content of the prepared microspheres was determined on a Moisture Balance (Mettler PM480 Delta Range). Determinations were done in triplicate.

6. Particle size and size distribution of microspheres

The particle size and size distribution of the polymers and microsphere formulations were determined by microscopy method. Samples of the microspheres were dispersed in normal saline containing 0.1% Tween 80 and photographed under a light microscope on which an ocular micrometer and a light camera are mounted (MT3300EXII, Microtrac-Bel, Japan). Hundred mL normal saline solution containing 0.1 mL Tween 80 was used to prepare the samples of microspheres to be mounted on the microscope. Approximately 1–2 drops of the solution was placed on the microscope slide and 100 mg of microspheres were dispersed carefully in the solution. Approximately 200 microspheres were counted and the mean diameter determined.²³

7. Powder X-ray diffraction (PXRD) study

Powder samples of the plain API, polymers and metformin-loaded microspheres were subjected to PXRD studies on an X-ray diffractometer (Rigaku Miniflex 600, Japan). The following conditions were used: a slit-detector Cu K α radiation source (30 kV, 15 mA, $\lambda = 0.15418$ nm), 2θ scan range was 3–35° and a scan rate of 4°/min under ambient temperature. This was carried out to detect any changes in the crystallinity of the API in the microsphere formulations.

8. Differential scanning calorimetry (DSC)

To further investigate the presence of any interaction between the polymers and the API, the thermal transition of the plain API, polymers and drug-loaded microspheres were assessed by DSC (PerkinElmer, USA apparatus). The DSC was calibrated using indium as a reference standard

(5 mg, 99.999% pure, onset at 156.6°C) and then the thermal behavior of the samples was measured. Approximately 5 mg of each sample was placed in a sealed aluminum pan and heated from 25 to 230°C at a scanning rate of 10°C/min under a nitrogen flow of 20 mL/min.

9. In vitro drug release

The drug release profile from the microspheres was measured using a Dissolution Tester (USP ELECTRO-LAB TDT-08L). A volume of 900 mL of 6.8 pH phosphate buffer was used as the dissolution medium. A bath temperature of $37 \pm 2^\circ\text{C}$ and basket rotation of 100 rpm was maintained throughout the period of measurement.²⁴ A microsphere formulation equivalent of 100 mg metformin hydrochloride was used. Samples (5 mL) were withdrawn at time 0, 5, 10, 15, 30 and 60 min and then at 1 h intervals for 9 h and at 24 h intervals for five days. Each withdrawal was replaced by a fresh 5 mL phosphate buffer solution. The samples withdrawn were filtered through a 0.45 μm membrane filter and then the drug content in each withdrawn sample was determined on a UV-Visible Spectrophotometer (SPECORD 200 Analyticjena) at 233 nm. Determinations were done in triplicate.

10. Mechanism of drug release from microspheres

The mechanism of metformin release was determined by analyzing the drug release data with the zero order kinetic, first order kinetic, Higuchi model, Hixon-Crowell and Korsmeyer-Peppas equations. The constants of release kinetic and coefficient of correlation (r^2) were obtained from slopes of plots by linear regression analysis. However, in order to determine the mechanism of drug release, the release data was fitted in a Korsmeyer-Peppas equation:^{21,2}

$$\text{Log (Mt/Mf)} = \text{Log } k + n \text{Log } t \quad (2).$$

This equation describes drug release behavior from polymeric systems. Mt is the amount of drug release at time t, Mf is the amount of drug release after infinite time; k is a release rate constant incorporating the structural and geometric characteristics of the dosage form and n is the diffusional exponent, which indicates the mechanism of drug release. For a cylinder shaped matrix, the value of $n = 0.45$ indicates Fickian (case I) release; > 0.45 but < 0.89 for non-Fickian (anomalous) release; and > 0 indicates a super case II type of release. The case II mechanism refers to the erosion of the polymer and anomalous transport (non-Fickian) refers to a combination of both diffusion and erosion controlled drug release. The mean dissolution time (MDT) is a more accurate drug release rate than the tx%. The equation is used to characterize drug release rate from the dosage form and the retarding efficiency of the polymer. Values of MDT can be calculated from dissolution data using the equation:

$$\text{MDT} = (n/n + 1)k - 1/n \quad (3),$$

where n is the release exponent and k is release rate constant. A higher value of MDT indicates a higher drug retaining ability of the polymer.²

11. Mucoadhesive properties

The mucoadhesive properties of the formulations were assessed *ex vivo* according to the method used by Odeniyi et al.² An ileum segment of a butchered goat, freshly incised, was obtained from the slaughterhouse, Faculty of Agriculture, University of Ibadan, Nigeria. Approximately 100 mg of each microsphere formulation was attached to the base of an aluminum probe, fixed to the mobile arm of a Texture Analyzer (TA-XT2i, Stable Micro Systems, Surrey, UK). The attached sample was lowered slowly at a rate of 0.1 mm/s to make contact with the ileum. A contact force of 0.25 N between the ileum and the microsphere was maintained for 5 min. The aluminum probe was withdrawn at the slow rate of 0.1 mm/s. The force required to detach the microsphere from the intestine was recorded as a measure of the bioadhesion. Determinations were done in triplicate.

12. Statistical analysis

The results obtained were subjected to statistical analysis using ANOVA, followed by posthoc Tukey's test, where more than two sets of data were obtained, to determine the level of significance (p-value) of an effect or the difference between means. Parameters that are significant at 95% confidence were considered significant or different at $p = 0.05$.

Discussion

FT-IR spectroscopy

Possible drug-polymer interaction was studied by FT-IR spectroscopy; the infrared spectra are shown in Fig. 1a–e. There was no difference in the bands shown by the plain drug and when formulated with each of the polymers. This indicates that the functional groups were not altered in the formulations due to interaction between metformin hydrochloride and the polymers. However, reduction in the intensities of the bands of metformin was observed in all the formulations; this is due to the reduction in crystallinity of the API by the amorphous polymers.



Fig. 1a. FT-IR of metformin

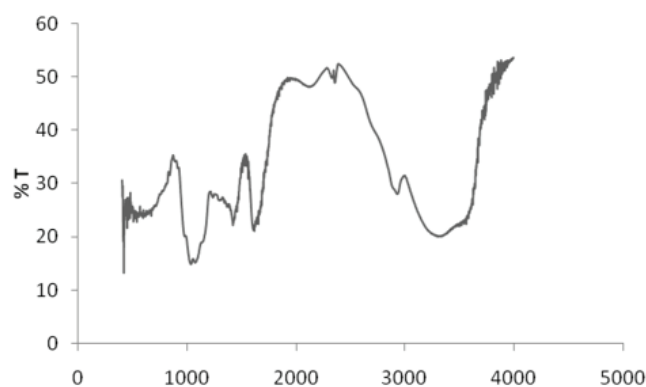


Fig. 1b. FT-IR of ET

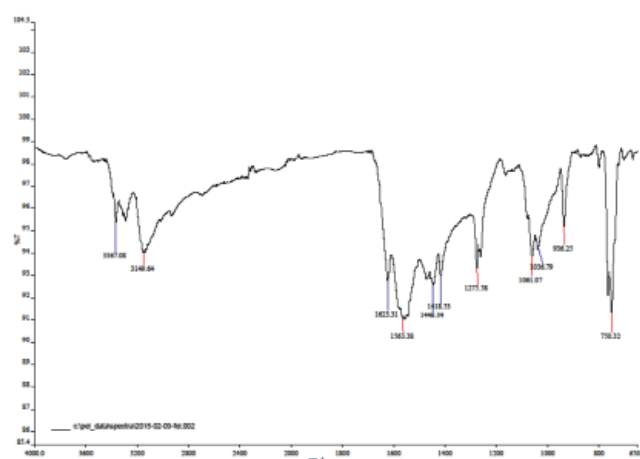


Fig. 1c. FT-IR spectra of FE

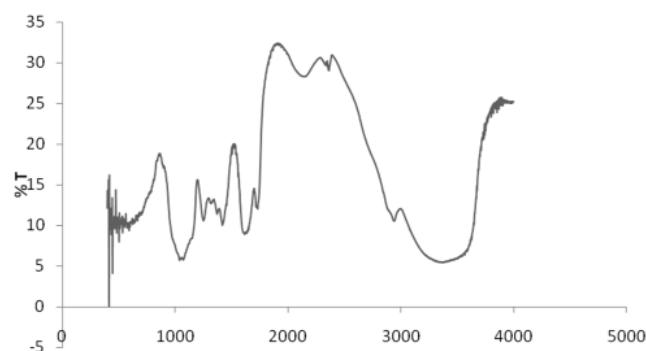


Fig. 1d. FT-IR of CD



Fig. 1e. FT-IR of FC

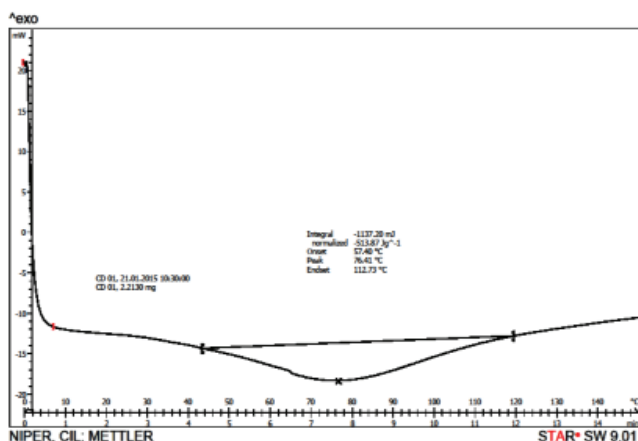


Fig. 2a. Differential scanning calorimetry of cedrela gum

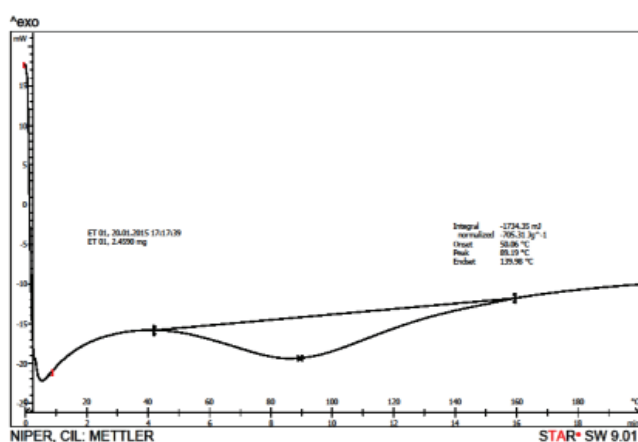


Fig. 2b. Differential scanning calorimetry of enterolobium gum

Drug-polymer interaction

To further study API-polymer interaction, the behavior of the individual polymers and microsphere formulations was studied by Differential Scanning Calorimetry (DSC) and Powder X-ray Diffraction (PXRD).

DSC is an analytical technique used to determine the quantity of heat either absorbed or released when a material undergoes physical or chemical changes.²⁶ Fig. 2a–b shows the thermal behavior of cedrela and enterolobium gums.

There was a wide range of temperatures between the onset and endset temperatures for the 2 polymers (Table 1); this indicates the amorphous nature of the gums. There was a sharp endothermic peak observed for metformin at 222.452°C, indicating the presence of a crystalline drug and the relative purity of the metformin drug sample compared to the polymers. Lower peaks were obtained for the polymers; this showed the amorphous nature of the gums and HPMC. The relatively low values obtained for the enthalpy change is attributable to the absence of the long chain of amylopectin molecules as found in starches.²⁷ Furthermore, the microsphere formulations generally reduced the intensity of bands

of metformin; this is due to the reduction of crystallinity of the API when being loaded into the polymers.

The PXRD studies (Fig. 3a and 3b) show the diffractograms of metformin, the polymers and the microsphere formulations. The polymers generally showed the broad peaks of a halo pattern which indicates an amorphous nature. The diffraction patterns of amorphous solids consist of broad peaks often referred to as an amorphous halo because amorphous systems have little long-range order.²⁸ Metformin displayed crystallinity by showing peaks at 2θ of 12, 13, 18, 22, 24, 25, 32, 34. Similar peaks were produced by the microsphere formulations but with significantly reduced intensity. This can be attributed to

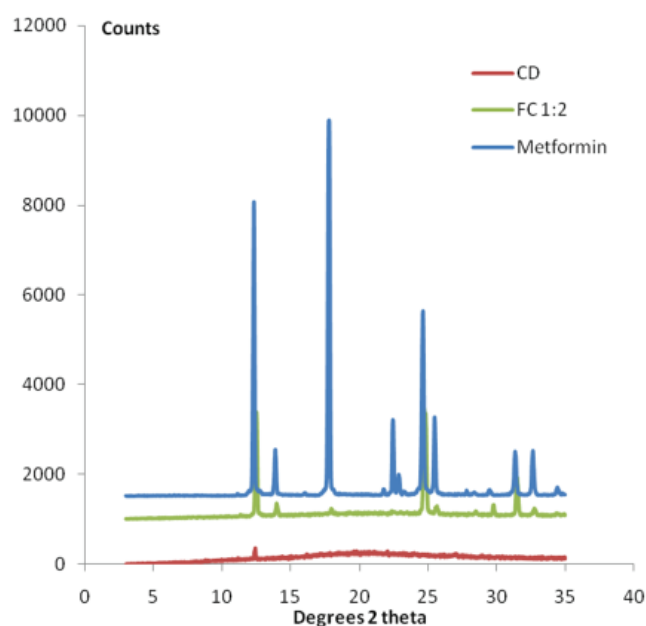


Fig. 3a. PXRD diffractograms of metformin, cedrela gum and FC at ratio 1 : 2

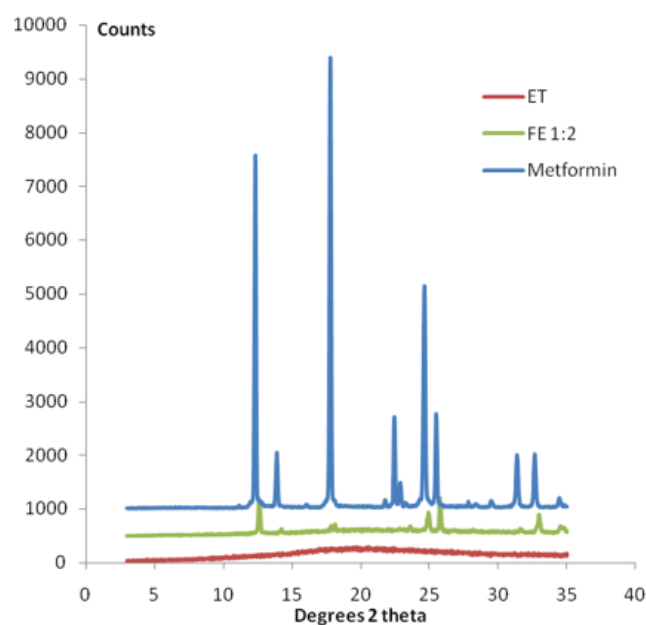


Fig. 3b. PXRD diffractograms of metformin, enterolobium gum and FE at ratio 1 : 2

Table 2. Properties of the microspheres

Formulations	Yield (%)	Actual drug content (mg)	Theoretical drug content (mg)	Drug entrapment efficiency (%)	Mean particle size (μm)	Moisture content (%)	Bioadhesion (peak detachment force) (N)
FC1	92.00 $\pm$ 12.23	380.00 $\pm$ 2.3	500	76.01 $\pm$ 2.11	80.10 $\pm$ 0.02	8.21 $\pm$ 0.15	1.22 $\pm$ 0.12
FC 2	96.70 $\pm$ 15.12	387.40 $\pm$ 2.5	500	77.48 $\pm$ 1.45	84.20 $\pm$ 0.34	8.78 $\pm$ 2.1	1.22 $\pm$ 0.23
FC 3	97.70 $\pm$ 15.11	390.10 $\pm$ 2.1	500	78.02 $\pm$ 0.25	81.40 $\pm$ 0.25	10.24 $\pm$ 5.21	1.25 $\pm$ 0.15
FC 4	94.40 $\pm$ 8.25	400.05 $\pm$ 2.0	500	80.01 $\pm$ 5.45	81.70 $\pm$ 0.22	10.20 $\pm$ 2.35	1.27 $\pm$ 0.14
FE1	96.80 $\pm$ 10.11	380.50 $\pm$ 1.6	500	76.10 $\pm$ 4.45	75.60 $\pm$ 0.15	8.96 $\pm$ 3.01	1.32 $\pm$ 1.45
FE2	92.50 $\pm$ 22.12	387.05 $\pm$ 1.8	500	77.41 $\pm$ 7.10	77.60 $\pm$ 0.17	6.80 $\pm$ 5.01	1.33 $\pm$ 0.91
FE 3	98.10 $\pm$ 10.50	392.50 $\pm$ 1.6	500	78.50 $\pm$ 1.55	77.00 $\pm$ 0.11	8.85 $\pm$ 11.50	1.38 $\pm$ 0.15
FE 4	98.90 $\pm$ 8.75	403.07 $\pm$ 1.5	500	80.61 $\pm$ 4.72	72.00 $\pm$ 0.13	9.65 $\pm$ 7.11	1.38 $\pm$ 0.15
FH 1	94.60 $\pm$ 9.45	381.15 $\pm$ 2.2	500	76.23 $\pm$ 0.35	78.80 $\pm$ 0.22	4.00 $\pm$ 0.01	1.31 $\pm$ 1.55
FH 2	95.10 $\pm$ 12.15	386.25 $\pm$ 2.2	500	77.25 $\pm$ 3.75	77.80 $\pm$ 1.25	3.880 $\pm$ 6.01	1.31 $\pm$ 0.55
FH 3	97.50 $\pm$ 10.11	397.45 $\pm$ 3.1	500	79.49 $\pm$ 2.28	77.50 $\pm$ 0.45	3.98 $\pm$ 4.01	1.33 $\pm$ 0.55
FH 4	98.40 $\pm$ 8.55	404.15 $\pm$ 2.0	500	80.83 $\pm$ 2.69	77.80 $\pm$ 0.22	4.75 $\pm$ 7.55	1.35 $\pm$ 0.15

a dilution effect or decrease in crystallinity of the API after incorporation into microspheres. This is expected to be an advantage in the delivery of metformin from the dosage form because the conversion from the crystalline form of metformin to the amorphous form will enhance better dissolution.

Evaluation of the microspheres

Microspheres loaded with metformin were prepared by the W/O emulsion solvent evaporation technique. The polymers formed mucilaginous dispersion in water with good swelling properties. The percent yield of microspheres from each batch of the formulations ranged from 92.0 to 98.9% (Table 2). This shows that the solvent extraction method is suitable for the formulation of microspheres. It is also an indication that the polymers can form microspheres with the API.

The entrapment efficiency (EE) of the microsphere formulations is shown in Table 2. High values of 76.01 to 80.83% were obtained from the formulations. This indicates that the loaded API is efficiently embedded in the microspheres. EE also increased with polymer concentration, showing that more drug particles are entrapped as the polymer molecules in the formulation increase. There was no significant difference between the EE of the polymers.

The particle size of the polymers (Table 3) was significantly higher than that of the microspheres. A particle size range of between 72 and 84 μm was recorded for all the microsphere formulations (Table 2). This indicates

a reduction in particle size of the polymers after being formulated as microspheres which could be due to the increase in surface area of the materials. Increased surface area is a major requirement for mucosal surface adhesion.²⁹ The marked reduction in particle sizes of the formulation indicates an increase in area-to-volume ratios of the particles; hence the rate of release of the drug from microsphere formulations will also increase. Furthermore, water absorption into smaller particles will be faster because of the shorter distance between the surface and center of the particles; hence there will be an increased rate of swelling. The size and distribution was adequate for optimum absorption across the mucosal layer, the stirring and speed employed in the formulation process probably accounted for the narrow range of particle sizes.¹⁷

Representative images from the morphological studies of the polymers and microspheres by Scanning Electron Microscopy are presented in Fig. 4d–e. The microspheres were almost spherical with some aggregations. The aggregation between spheres could be due to the adhesive property of the gums. Less aggregation was observed in formulations containing enterolobium gum and HPMC. The microspheres became more spherical with increasing polymer concentration; this is attributable to the polysaccharide composition and gelling properties of the polymers.

Low moisture loss of 3.88–10.24% was obtained in all the formulations (Table 3). This showed that no significant quantity of water was present in the microspheres after formulation.

Table 3. Particle size and size distribution of polymers

Polymer	Particle size (μm)			
	D ₁₀	D ₅₀	D ₉₀	span
Cedrela gum	138.30	436.00	641.40	1.15
Enterolobium gum	120.90	274.70	530.50	1.49
HPMC	130.00	145.00	510.20	2.62

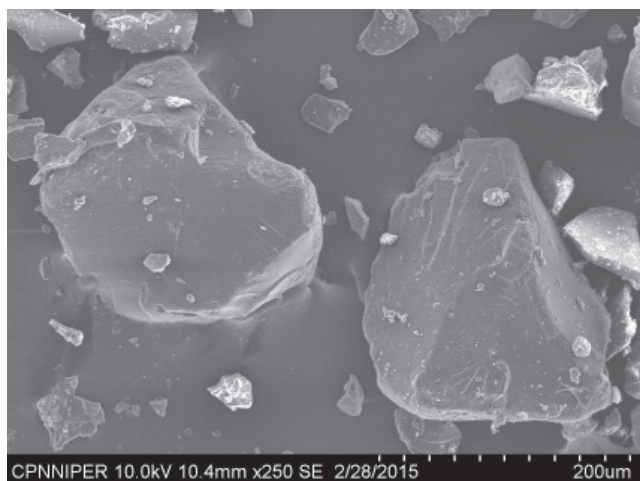


Fig. 4a. SEM images of cedrela gum

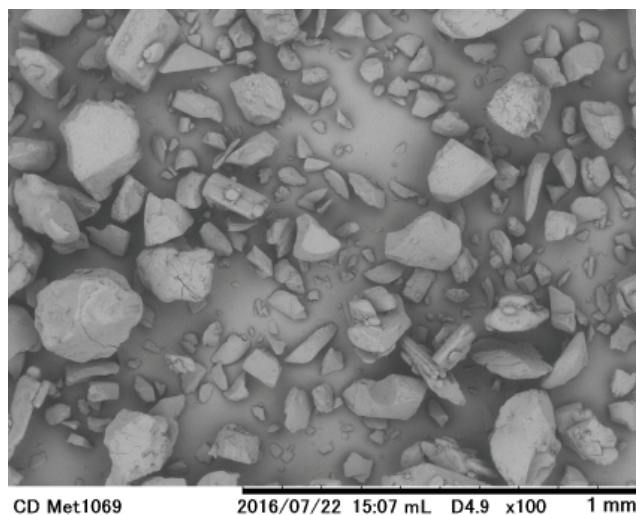


Fig. 4d. SEM images of FC

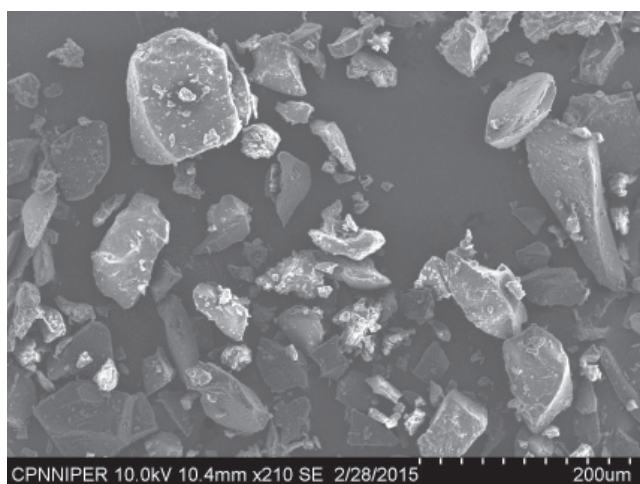


Fig. 4b. SEM images of enterolobium gum

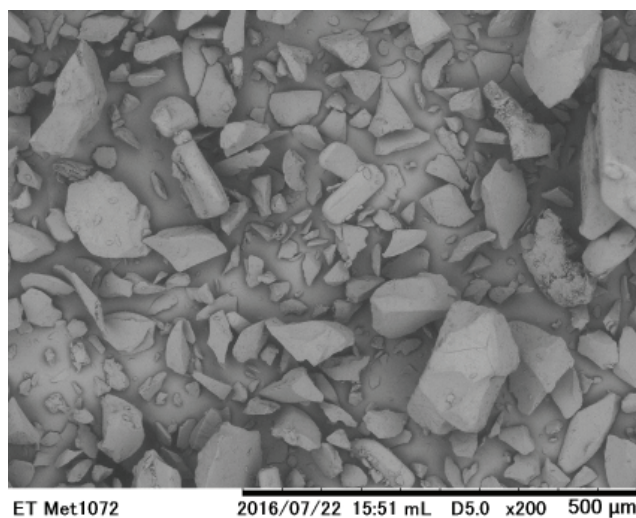


Fig. 4e. SEM images of FE

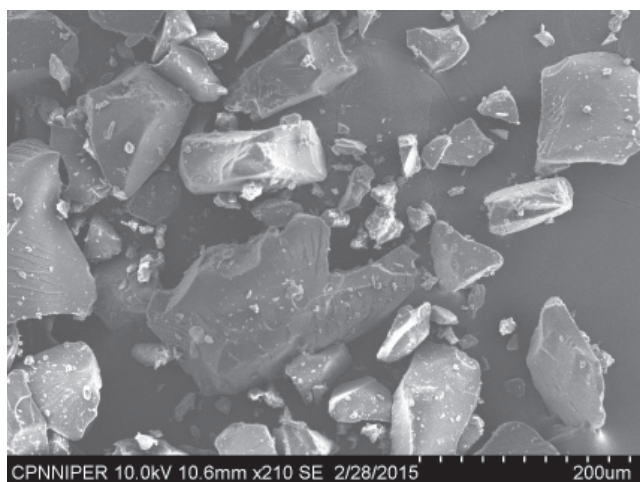


Fig. 4c. SEM images of HPMC

Release of the drug from microspheres

There was an initial burst release within the 30 min. Thereafter, a constant drug release was observed over 5 h, followed by a much slower release up to day 5 of the dissolution experiment (Fig. 5a–c). Formulations containing enterolobium gum exhibited the highest percentage of drug release, the ranking order was FE > FH > FC, with no significant difference. Furthermore, the mean dissolution time was highest for FE, with a similar ranking order. This indicates that formulations containing cedrela and enterolobium gums are capable of retaining the loaded API for the same period as with HPMC.

To study the mechanism of metformin release from the microspheres, drug release data was fitted into various mathematical models (zero order kinetic, first order kinetic, Higuchi model, Hixson-Crowell and Korsmeyer-Peppas equations) to obtain the coefficient of correlation (r^2) and n values. The values obtained are presented in Table 4.

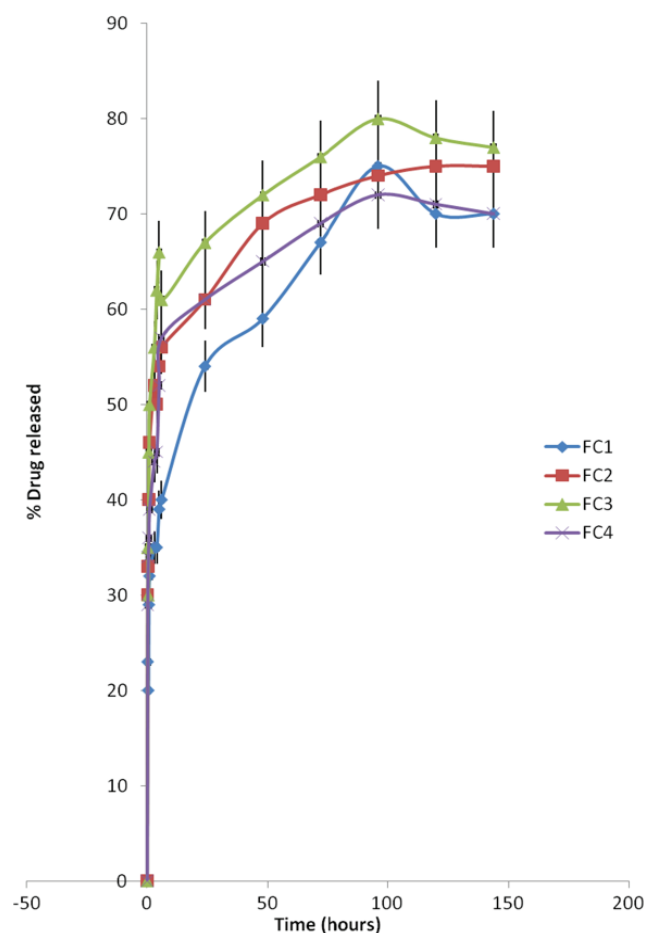


Fig. 5a. Drug release profile of microsphere formulations containing cedrela gum

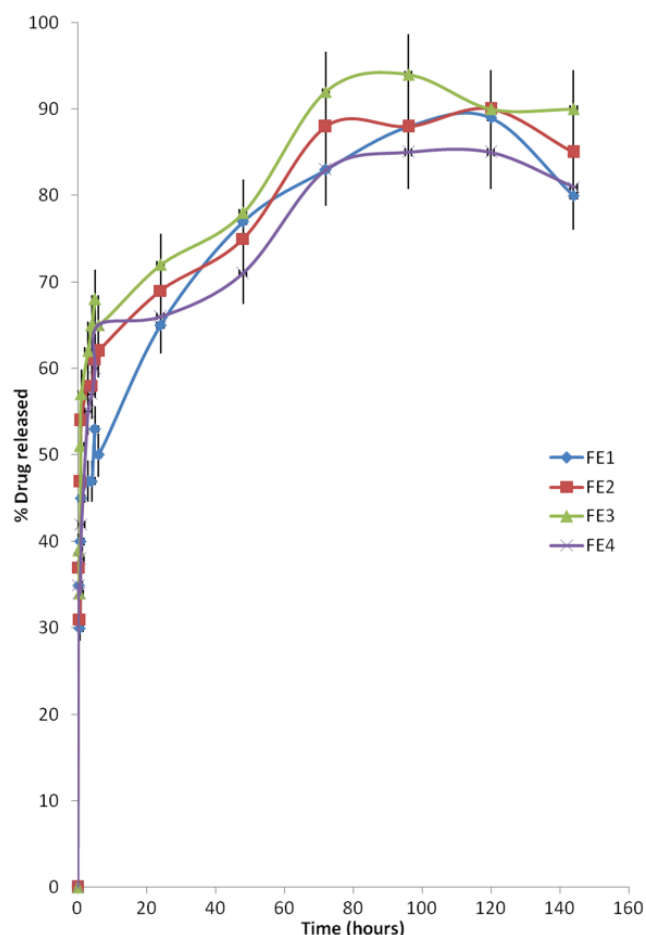


Fig. 5b. Drug release profile of microsphere formulations containing enterolobium gum

The values of n from the Korsmeyer-Peppas model, which is capable of describing the mechanism of drug release from polymeric systems, were considered.²⁵ When n is 0.43 or less, it indicates release is by diffusion mechanism. When n is 0.85, the mechanism of release is swelling controlled and when n is between 0.43 and 0.85, the mechanism of drug release is by both diffusion and swelling controlled mechanisms; this is termed anomalous.³⁰ In all the formulations, the values of n were less than 0.43; according to the data obtained, the main mechanism of metformin release from the microspheres is diffusion. Furthermore, all the formulations except FC3 and FC4 had the highest values of r^2 in the Korsmeyer-Peppas equation, while FC3 and FC4 had the highest r^2 values in first order kinetic. This shows that the release of metformin in all formulations except FC3 and FC4 was not concentration dependent while that of FC3 and FC4 depends on the concentration of the polymers. The model-independent dissolution parameters are presented in Table 5. From these parameters, the Mean Dissolution Time (MDT) for the formulations was obtained. The data showed that the formulations were able to sustain metformin for a period ranging from 43 to 46 h, indicating the possibility of reducing the dosing frequency of metformin.

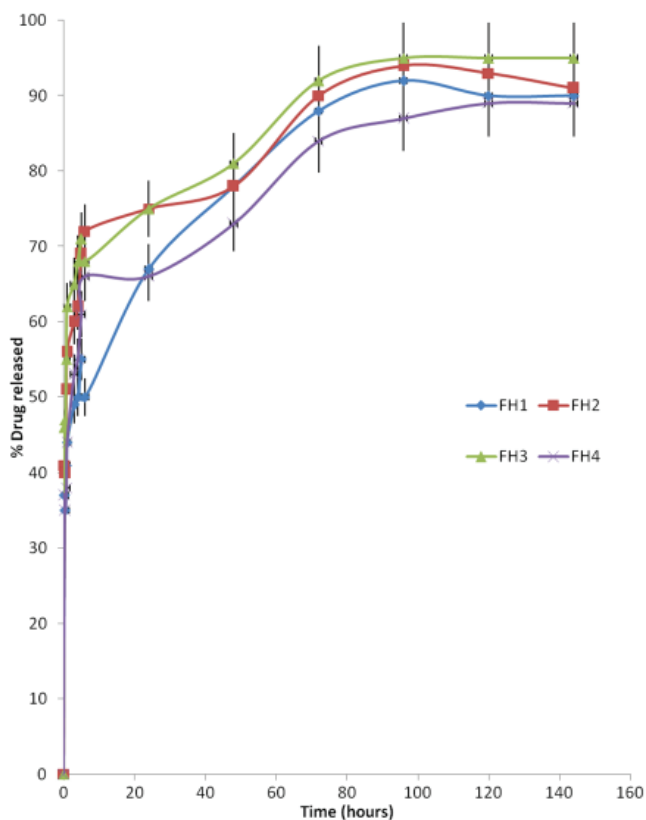


Fig. 5c. Drug release profile of microsphere formulations containing HPMC

Table 4. In vitro release kinetics of microsphere formulations

Formulation code	Zero-order		First-order		Higuchi		Hixson-Crowell		Korsemeyer-Peppas		
	r^2	k_0	r^2	k_1	r^2	k_H	r^2	k_{HC}	N	r^2	k
FC1	0.895	0.671	0.962	0.019	0.962	7.577	0.948	0.004	0.186	0.988	29.53
FC2	0.833	0.719	0.956	0.284	0.913	8.351	0.913	0.009	0.120	0.973	42.84
FC3	0.745	0.755	0.958	0.392	0.836	8.870	0.835	0.010	0.107	0.924	48.04
FC4	0.821	0.684	0.981	0.244	0.910	7.950	0.916	0.008	0.125	0.975	39.97
FE1	0.879	0.813	0.926	0.250	0.952	9.294	0.968	0.010	0.155	0.981	41.06
FE2	0.839	0.839	0.914	0.389	0.912	9.704	0.912	0.010	0.127	0.961	48.21
FE3	0.820	0.875	0.905	0.509	0.895	10.158	0.893	0.010	0.119	0.951	52.12
FE4	0.823	0.799	0.956	0.341	0.903	9.248	0.901	0.010	0.131	0.961	45.35
FH1	0.912	0.859	0.913	0.270	0.973	9.766	0.981	0.010	0.159	0.991	42.48
FH2	0.839	0.884	0.918	0.526	0.913	10.266	0.906	0.010	0.116	0.968	53.39
FH3	0.875	0.907	0.855	0.754	0.937	10.548	0.925	0.011	0.104	0.976	57.40
FH4	0.871	0.837	0.940	0.341	0.935	9.601	0.924	0.010	0.139	0.974	45.40

Mucoadhesive properties

Mucoadhesion is a measure of the strength of contact between the drug delivery system and mucosal surface. The formulations exhibited good mucoadhesion characteristics in this ranking order: FE > FH > FC, with no significant difference (Table 3). There was an increase in peak detachment force with the increasing concentration of polymer in all the formulations, which agrees with the previous report that an increasing concentration of bioadhesive polymer is capable of increasing the binding potential.⁵ The polymers, being hydrophilic, absorb water, swell and these enhance mucoadhesion with the mucosal layer. The swelling led to formation of bonds and a spatial network between the mucous membrane and the adhesive polymer in the microspheres. Also, functional groups such as the carboxyl group, present in the gums, are capable of forming hydrogen bonds with the mucin molecules, leading to mucoadhesion.

Conclusion

A mucoadhesive microsphere drug delivery system of metformin hydrochloride was successfully formulated from native *Enterolobium cyclocarpus* and *Cedrela odorata* plant gums. The mechanism of drug release from the microspheres was diffusion. All the formulations exhibited good mucoadhesion properties and a Mean Dissolution Time (MDT) of 43–65 h, which is suitable for a reduced dosing frequency. These native gums may be considered for intestinal drug delivery.

The obtained results are indicative of the need of non-surgical treatment in the group of patients with a past history of myocardial infarction. Continuation of randomized research on a larger group of patients is necessary to obtain a reliable evaluation of therapy effects on the periodontal status.

Table 5. Dissolution parameters of formulations

Formulation code	$t_{25\%}$ (h)	$t_{50\%}$ (h)	$t_{75\%}$ (h)	$t_{90\%}$ (h)	MDT (h)
FC1	0.408	17.029	151.100	403.248	61.23
FC2	0.011	3.635	107.529	493.138	63.26
FC3	0.002	1.451	63.650	348.458	63.11
FC4	0.023	6.000	154.072	663.045	65.40
FE1	0.041	3.551	48.215	155.808	52.51
FE2	0.006	1.332	32.236	135.079	52.65
FE3	0.002	0.706	21.023	96.685	48.90
FE4	0.011	2.109	46.716	188.133	57.51
FH1	0.035	2.791	35.873	113.090	46.35
FH2	0.001	0.568	18.680	89.817	48.63
FH3	0.000	0.266	13.009	74.787	43.07
FH4	0.014	2.001	36.946	137.072	52.38

References

- Smart JD. The basics and underlying mechanisms of mucoadhesion. *Adv Drug Del.* 2005;57:1556–1568.
- Odeniyi MA, Khan NK, Peh KK. Release and mucoadhesion properties of diclofenac matrix tablets from natural and synthetic polymer blends. *Act Pol Pharm Drug Res.* 2015;72:559–567.
- Verbeken D, Dierckx S, Dewettinc K. Exudate gums: Occurrence, production and application. *Appl Microb Biotech.* 2003;6:10–21.
- Ricon F, Munoz J, Leon de Pinto G, Alfaro MC, Calero N. Rheological properties of *Cederela odorata* gum exudates aqueous dispersions. *Food Hydrocolloids.* 2009;23:1031–1037.
- Odeniyi MA, Babalola AO, Ayorinde JO. Evaluation of Cedrela gum as a binder and bioadhesive component in ibuprofen tablet formulations. *Braz J Pharm Sci.* 2013;49:95–105.
- Hughes CE, Stewart JL. A quick guide to useful nitrogen fixing trees from around the world. *N.F.T.A.* 1990;90:77.
- Janzen DH. *Enterolobium cyclocarpum*. In: Costa Rican Natural History. Ed: Janzen D.H. Univ. Press, Chicago 1983;816.
- Gladys L, Maritza M, Aida L, Carlos R, Edgar C. Chemical and ¹³CNMR studies of *Enterolobium cyclocarpum* gum and its degradation products. *Phytochem.* 1994;37:1311–1315.
- Oliveira JD, Silva DA, Paula RCM, Feitosa JPA, Paula HCB. Composition and effect of salt on rheological and gelation properties of *Enterolobium* gum exudate. *Int J Biol Macromol.* 2001;29(1):35–44.
- Akpabio EI, Ekong US, Uwah TO, et al. Formulation and evaluation of sustained release tablets using *Lesianthera Africana* gum. *Nig J Pharm Appl Sci Res.* 2016;5(2):41–48.
- Adedokun MO, Ayorinde JO, Odeniyi MA. Compressional, mechanical and release properties of a novel gum in paracetamol tablet formulations. *Curr Iss Pharm Med Sci.* 2014;27(3):187–194.
- Emeje M, Nwabunike P, Ismi C, et al. Isolation, characterization and formulation properties of a new plant gum obtained from *Cissus refescence*. *Int J Green Pharm.* 2009; 3:16–23.
- Odeniyi MA, Takeuchi H. Design and evaluation of cedrela gum based microparticles of theophylline. *Ars Pharmaceutica.* 2014;55(1):30–36.
- Sharma HK. Preparation and in vitro permeation study of nasal gel of oxytosin with natural mucoadhesive substances. Dissertation 2003, Jadavpur University Kolkata.
- Hundal RS, Inzucchi SE. Metformin: New understandings, new uses. *Drugs.* 2003;6(18):1879–1894.
- Garry G, Graham JP, Manit A, et al. Clinical pharmacokinetics of metformin. *Clin Pharmacokinet.* 2011;50(2):81–98.
- Banarjee P, Deb J, Roy A, Ghosh A, Chakraborty P. Fabrication and development of pectin microsphere of metformin hydrochloride. *ISRN Pharmaceutics.* 2012, doi: 10.5402/2012/230621
- Saeed RN, Muhammad AA, Muhammad NK, Mohamed AH, Nadeed KM, Azmi AH. Formulation development of metformin tablet and its comparative in-vitro study with different brands in Pakistan. *Int J Pharm Sci Rev Res.* 2013;19(2):12–17.
- Berressem P. The birth of new delivery systems. *Chem Br.* 1999;35(2):29–32.
- Bamiro OA, Sinha VR, Kumar R, Odeku OA. Characterization and evaluation of *Terminalia randi* gum as a binder in carvedilol tablet formulation. *Acta Pharm Sci.* 2010; 52:254–262.
- Adetunji OA, Odole MO. Assessment of the suspending properties of naturally occurring *Entandophragma angolense* gum in oral sulphamethoxazole suspension. *J Chem Pharm Res.* 2015;7:870–878.
- Ayorinde JO, Odeniyi MA, Balogun-Agbaje O. Formulation and evaluation of oral dissolving films of amlodipine besylate using blends of starches with hydroxypropyl methyl cellulose. *Polym Med.* 2016;46(1):45–51.
- Tayade PT, Kale RD. Encapsulation of water-insoluble drug by a cross-linking technique: Effect of process and formulation variables on encapsulation efficiency, particle size and in vitro dissolution rate. *AAPS. PharmSci.* 2004;6:112–119.
- British Pharmacopoeia 2010, Volume I, Her Majesty's Stationery Office, London
- Dash S, Murthy PN, Nath L, Chowdhuri P. Kinetic modeling on drug release from controlled drug delivery systems. *Act Pol Pharm-Drug Res.* 2010;67(130):217–223.
- Othman N, Azahari NA, Ismail H. Thermal properties of polyvinyl alcohol (PVOH)/corn starch blend film. *Maylasian Polym J.* 2011;6(6):147–154.
- Ai Y, Gong L, Reed M, Huang J, Zhang Y, Jane J. Characterization of starch from bamboo seeds. *Starch.* 2016;68:131–139.
- Tiwari M, Chawla G, Bansal AK. Quantification of olanzapine polymorphs using powder X-ray diffraction technique. *J Pharm Biomed Anal.* 2007;43(3):865–872.
- Amaro MI, Tajber L, Corrigan OI, Healy AM. Co-spray dried carbohydrate microparticles: Crystallization delay/inhibition and improved aerosolization characteristics through the incorporation of hydroxypropyl- β -cyclodextrin with amorphous raffinose or trehalose. *Pharm Res.* 2015;32:180–195.
- Szekalska M, Winnicka K, Czajkowska-Koanik A, Sosnowska K, Amelian A. Evaluation of alginate microspheres with metronidazole obtained by spray drying technique. *Act Pol Pharm Drg Res.* 2015;72(3):569–578.

Film forming properties of *Cissus pulpunea* (Guill and Perr) and *Irvingia gabonensis* (O'Rorke) gums

Tolulope Omolola Ajala^{1,B–F}, Hope Idemudia Olaiya^{2,B,C,F}, Oluwatoyin Adepeju Odeku^{2,A,C,E,F}

¹ Department of Pharmaceutics and Industrial University of Ibadan, Ibadan, Nigeria

² Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;

D – writing the article; E – critical revision of the article; F – final approval of article

Polymers in Medicine, ISSN 0370-0747 (print), ISSN 2451-2699 (online)

Polim Med. 2017;47(1):25–33

Address for correspondence

Tolulope Omolola Ajala

E-mail: tolulola1721@gmail.com

Funding sources

none declared

Conflict of interest

none declared

Acknowledgements

The authors acknowledge Mr Bukola Omoniyi for his assistance during the experiments.

Received on January 6, 2017

Revised on March 19, 2017

Accepted on July 24, 2017

Abstract

Background. Natural polymers such as gums have gained attention in drug delivery systems due to their availability, compatibility and degradation under natural and physiological conditions.

Objectives. The aim of the present study was to investigate the film forming properties of gums obtained from the stem of *Cissus polpunea* (Guill and Perr) and the seed of *Irvingia gabonensis* (O'Rorke).

Material and methods. Gums were extracted from the relevant plant parts and characterized using functional, proximate and elemental properties. Films were prepared by the casting method using gum concentrations of 1–4% w/v and varied with propylene glycol (PG). The films were assessed through physical observation, thickness, swelling power and moisture sorption effects using the relative humidity of 0, 27, 43, 57, 75 and 90%. The gum yielding optimal film properties was used as coating material in ibuprofen tablet formulations. The mechanical and release properties of the tablets were determined.

Results. The functional and proximate properties of gums showed a similarity in the majority of the parameters, but significant ($p < 0.05$) variation existed in their solubility, while elemental assessment revealed the absence of toxic metals. Generally, the films were homogenous, opaque and demonstrated high swelling power in phosphate buffer, which was pH-dependent. Moisture sorption properties of the gums increased with the increase in relative humidity in the order HPMC < *Cissus* < *Irvingia*. Film-coated ibuprofen tablets showed higher mechanical properties and disintegration and dissolution times compared with uncoated tablets.

Conclusions. *Cissus* and *Irvingia* gums have demonstrated acceptable functional, proximate and elemental properties. Film-coated ibuprofen tablets showed higher mechanical and release properties than was the case in uncoated tablets.

Key words: *Cissus polpunea* gum, *Irvingia gabonensis* gum, ibuprofen tablet, film and coating properties

DOI

10.17219/pim/76059

Copyright

© 2017 by Wrocław Medical University

This is an article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

Polymers are widely used in drug delivery systems as film-forming materials for coating tablets either to protect the core of the tablet against degradation, mask taste or for sustained and controlled release dosage forms.¹ Natural polymers have gained attention as a drug delivery system due to their availability, compatibility and degradation under natural and physiological conditions.²

Cissus gum is obtained from the stem and roots of *Cissus pulpunea* (Guill and Perr), family *Ampelidaceae*. *Cissus* is a genus of approximately 350 species of woody vines in the grape family *Amplidaceae* (*Vitaceae*). *Cissus* gum is traditionally used in the preparation of soup amongst the Idomas of Benue State and the Igalas of Kogi State of Nigeria.³ It has also been used as a foam stabilizer in preparing 'akara' balls.⁴ On the other hand, *Irvingia* gum is obtained from *Irvingia gabonensis* (O'Rorke) Bail (family *Irvingiaceae*), commonly known as African mango or 'bush mango', which is a tree of 15–40 m with a slightly buttressed hole.⁵ The kernels from the tree are called oilseeds and have various local names: in Nigeria, such as 'ogbono' in Ibo and 'apon' in Yoruba.⁶ The kernels are ground with a pestle and mortar or on a stone into a paste or cake called 'dika bread', which is used as a soup, stew or sauce additive, for flavoring and thickening.⁷ The kernels are also highly valued for the slimy consistency they produce because of the presence of mucilage. The mucilage has been reported to be a binding agent in tablet formulation, especially when slower disintegration and dissolution rates are desired⁸ and the emulsifying and suspending properties have also been studied.⁹

In a recent study, some material properties of both gums (*Cissus* and *Irvingia*) such as porosity, phase transition, X-ray diffraction patterns, gelatinization temperature, heat of gelatinization, mean particle size and specific surface area were assessed and the results showed acceptable excipient potentials; furthermore, the gums successfully served as polymers in microbead design.¹⁰ In addition, unpublished data also showed that *Cissus* and *Irvingia* gums lacked toxicity against different cell lines. However, the prospect of these gums in film formation and coating of tablets for immediate release has not been evaluated. Consequently, the film-forming properties of the gums in comparison with hydroxypropyl methyl cellulose (HPMC – a standard polymer used in the food and pharmaceutical industry) have been evaluated and optimal concentration of the films was used for coating ibuprofen tablet formulation.

Material and methods

Material

The materials used included: ibuprofen powder obtained from Vital Medix Nigeria Limited (Ibadan, Nigeria), HPMC E5 Premium LV was obtained from Colorcon

limited (Flagship House, Kent, England), propylene glycol (PG) was procured from BDH Chemicals Limited (Poole, England). Kernels of *Irvingia gabonensis* were purchased from Ojoo market in Ibadan (South Western Nigeria) while the stems of *Cissus pulpunea* were purchased from Bode market also in Ibadan (South Western Nigeria).

Harvesting and extraction of *Irvingia gabonensis* gum

Irvingia gabonensis kernels were harvested from the fruit by dehulling, and extraction was done using an established method.⁸ Then, dried kernels of *Irvingia gabonensis* (without the seed coats) were powdered using a laboratory mill and then macerated in petroleum ether for 24 h, and then strained through a calico cloth to obtain particulate-free slurry. The extract separated from the residue was repeatedly soaked in petroleum ether until there was no more fat obtained in the petroleum ether. The fat-free residue was hydrated in chloroform water (double strength) for 5 days and *Irvingia* gum was precipitated with ethanol. The precipitated gum was washed with diethyl ether and dried in a hot air oven (Model 77-9083, Techmel&Techmel, China) at 50°C for 48 h. The dried gum was pulverized using a porcelain mortar and pestle. The fine particles were screened through a stainless steel sieve (250 µm) and stored in an airtight container in a dessicator prior to use.

Harvesting and extraction of *Cissus pulpunea* gum

The stems of *Cissus pulpunea* were cut by local farmers, cleaned, assembled and sold in a local market. *Cissus* stems were procured and prepared for extraction by scraping the stems with a knife to remove the outer layer of the stem. The scraped stems were washed in clean water and chopped into tiny bits ready for gum extraction. The chopped strips were soaked in chloroform-water double strength for 48 h. The gums were strained through calico cloth to remove extraneous materials and then precipitated with absolute alcohol. The precipitated gum was filtered and washed with diethyl ether. The purified gums were dried in a hot air oven (Model 77-9083, Techmel&Techmel, China) at 40°C for 48 h and then pulverized and kept in airtight containers.¹¹

Proximate analysis

The proximate constituents were determined using established procedures.¹² Standard AOAC methods were used to determine ash content (method 942.05), crude fiber (method 958.06), and moisture by the hot air oven method (method 925.09), crude protein was determined by routine semi-micro Kjeldahl method (Method 988.05) and (2003.06) in both *Irvingia* and *Cissus* gum powder.

Elemental constituents

The powdered gum (0.5 g) was weighed and placed in a 125 mL Erlenmeyer flask, which has been previously washed. Perchloric acid (4 mL), concentrated nitric acid (25 mL), and 2 mL concentrated sulphuric acid were added into the content in the flask. The contents were mixed gently at a low temperature on a hot plate in a perchloric acid fume cupboard. The heating was continued until dense white fumes appeared. Finally, the mixture was heated strongly (medium to high heat) for half a minute. This was allowed to cool, and then distilled water 40–50 mL was added and boiled for half a minute on the same plate at medium heat. Distilled water was added till the volume reached the 100 mL mark. The elemental constituents were then evaluated using Atomic Absorption Spectrophotometer (AAS, model 2500 Torontech, Inc., Toronto, ON, Canada).

Solubility

The solubility of the gums was done in water in accordance with the modified method described by Kaur et al.¹³ The gum (1.0 g) (w) was weighed into a 100 mL conical flask, 15 mL of distilled water was added and shaken slowly for 5 min, then transferred into a water bath and heated for 20 min at 80°C with constant stirring for 1 h. The mixture was then transferred into a pre-weighed centrifuge tube (w_1), distilled water (7.5 mL) was added and centrifuged (TDL-5, Mumbai, India) at 2200 rpm for 20 min. The supernatant was then carefully decanted into a pre-weighed dish (w_2), dried at 100°C to a constant weight (w_3) and cooled for 30 min. From the weight taken, the solubility in percentage was calculated using equation 1:

$$\text{solubility (\%)} = (w_2 - w_3 / w) \times 100 \quad (1).$$

Water absorption capacity

Water absorption capacity (WAC) was determined by placing the gum (2.5 g) in a weighed 50 mL centrifuge tube and 15 mL distilled water was added. Agitation on a vortex mixer was done for 2 min and later centrifuged (TDL-5, Mumbai, India) at 400 rpm for 20 min and the supernatant was decanted. The supernatant was discarded, the residue was weighed (w_1) and the absorbed drops of water were removed by drying at 100°C to a constant weight w_2 in an oven. Water absorption capacity was expressed as the weight of water bound by 100 g of sample and calculated using equation 2:

$$\text{WAC} = \{(w_1 - w_2) / 2.5\} \times 100 \quad (2).$$

Swelling index

The gum (5 g) was placed in each of 100 mL measuring cylinder and the volume occupied was noted (v_1). Distilled water (90 mL) was gradually added with agitation for 5 min and then made up to volume (100 mL). The mixture was allowed to stand for 24 h and the volume occupied after settling (v_2) was measured. The swelling index was computed as follows:

$$\text{swelling index} = v_2 / v_1 \quad (3).$$

Viscosity

The viscosities of aqueous dispersions (1, 2, 3 and 4% w/v) of each polymer (*Irvingia*, *Cissus* and HPMC) were determined using a Brookfield viscometer (RVDV-II+P, Middleboro, U.S.A) with spindle 4 and shear rate of 100 rpm at $25 \pm 2^\circ\text{C}$.

Preparation of films

A homogenous and clear dispersion (2, 3 and 4% w/v) of the gum was prepared by dispersing the weighed amount of the gum in distilled water and gradually heating with stirring (100 rpm) for 30 min at $70 \pm 5^\circ\text{C}$. The solution was then filtered through a calico cloth to remove undissolved particles and 20 mL was transferred to a petri dishes having 9 cm internal diameter. The petri dishes were placed on leveled surfaces for casting and then the films were dried in a hot air oven (Model 77-9083, Techmel&Techmel, China) at 50°C for 48 h. The films were carefully removed from the Petri dish and equilibrated at $25 \pm 2^\circ\text{C}$, 58% relative humidity for 24 h in a glass dessicator.

Determination of film thickness

The thickness of the films was measured with a micrometer screw gauge (sensitivity of 0.001 mm). Then measurements were taken at random locations on the film and the mean values were calculated.

Moisture sorption of gum and prepared films

Moisture sorption isotherm was determined by cutting the films into 25×10 mm. The dried films or powdered gums were placed inside a vacuum dessicator maintained at 0, 27, 43, 57, 75, and 90% relative humidity (RH) using super saturated solutions of different solutes kept at $25 \pm 2^\circ\text{C}$ and equilibrated for 14 days. The amount of water absorbed was determined by re-weighing the containers and the contents. The moisture sorption curve was obtained by plotting a graph of percentage moisture content vs percentage relative humidity.¹⁴

Percentage moisture sorption was calculated from the formula below:

$$\% \text{ moisture content} = \{(M_{\text{ae}} - M_{\text{be}}) / M_{\text{be}}\} \times 100 \quad (4),$$

where: M_{ae} = mass after equilibrium,
 M_{be} = mass before equilibrium.

Swelling index of dried film

The swelling index was determined by placing an accurately weighed (w) film with area of 1 cm^2 ($1 \times 1 \text{ cm}$) in a flask containing 250 mL of phosphate buffer pH 4.75, 6.0 and 7.4 at $37 \pm 2^\circ\text{C}$. Swollen samples were withdrawn from the medium and weighed (w_s) after the removal of excess surface water by light blotting with Whatman paper. The swelling index (%) was calculated as using equation 7 below:

$$\text{swelling index (\%)} = \{(w_s - w) / w\} \times 100 \quad (5).$$

Preparation of granules

Batches (300 g) of a basic formulation of ibuprofen (73% w/w), corn starch (9% w/w), PVP (2% w/w), sodium benzoate (1% w/w), magnesium stearate (2% w/w) and lactose (14% w/w) were dry-mixed for 5 min in a Kenwood planetary mixer (Model A120, Hobart Manufacturing Co, U.K). The PVP was prepared as mucilage and used to moisten the dry-mixed powder to produce granules. Massing was continued for 5 min and the wet masses were granulated by passing them manually through a number 12 mesh size ($1400 \mu\text{m}$), dried in a hot oven for 4 h at 50°C and then re-sieved through a number 16 mesh sieve ($1000 \mu\text{m}$). The granules were then stored in airtight containers.

Preparation of tablets

Tablets of $400 \pm 10 \text{ mg}$ were prepared from the granules by compressing them in an Erweka automated tableting machine (Model EP-1, Heusenstamm, Germany). Before compression, the depth of the die and punches were adjusted to produce the desired strength of the tablet. After this was achieved, the machine was then allowed to produce the tablets automatically for 30 min. After ejection, the tablets were stored over silica gel for 24 h to allow for elastic recovery and hardening.

Coating of tablets

Tablet coating was performed in a high efficiency coating machine (BG-80, Zhejiang, China) with 2 spray guns. Tablet cores were pre-heated to about 40°C in the coating pan. The spray gun was filled with 3% w/v *Cissus* coating dispersion and operated at a proper flow rate. The pan

was set into motion and coating solution was sprayed onto the falling cores under a suitable air pressure (30–35 psi). Upon completion of the seal coating, the air heater was switched off and the tablets were blown-dried for 20–25 min in the coating pan.

Uniformity of weight for tablets

Ten tablets were weighed individually and collectively using a Mettler electronic balance (Model FA2104A, United States) and the average weight was determined. The percentage deviation was then calculated from the average weight.

Tablet hardness and friability

The load (N) required to diametrically break the tablet was determined at room temperature using a DBK tablet hardness tester (Model EH01, Mumbai, India). The average readings for 10 tablets were taken as the crushing strength. The percentage friability of the tablets was determined with ten tablets using a DBK Friability test apparatus (Model 40FTA01, England) operated at 25 rpm for 4 min.

Disintegration and dissolution test

The disintegration time of the tablets was determined in distilled water at $37 \pm 0.5^\circ\text{C}$ using a DBK Disintegration rate test apparatus (40TDA01, Mumbai, India). The dissolution time of the tablet was determined using the DBK dissolution rate test apparatus (40DRV01, Mumbai, India) containing 900 mL of phosphate buffer pH 6.8 maintained at $37 \pm 1^\circ\text{C}$ at a paddle speed of 50 rpm. Samples (5 mL) of the dissolution medium were withdrawn at different time intervals and replaced with fresh (5 mL) dissolution medium at the same temperature. The samples were spectrophotometrically analyzed at a wavelength of 224 nm using a UV spectrophotometer (LAMBDA 12, Perkin Elmer GmbH, Urberlingen, Germany). The percentage drug released was plotted against time.

Data presentation and analysis

Most of the experiments were performed in triplicate, for film thickness, 10 readings per sample had to be taken at different points on the film surface while the number of tablets used for evaluating tablet properties vary depending on the type of test and specifications in official compendia. In addition, data has been presented as mean $\pm$ standard deviation and “n” indicated on each table. Statistical analysis was carried out using the analysis of variance (ANOVA). The difference between formulations was compared using Turkey Kramer’s multiple comparison tests. At 95% confidence interval, probability values less than or equal to 0.05 were considered significant.

Results and Discussion

Properties of gums

Irvingia and *Cissus* gums are natural polymers obtained from local edible sources in Nigeria and work is ongoing to fully characterize and determine their chemical structures. HPMC is a standard polymer which has been chemically characterized and is generally used as excipient in food and pharmaceutical industry. Moreover, pursuant to 21 CFR 5170-30, the use of HPMC food or pharmaceuticals is generally recognized as safe (GRAS).¹⁵ The functional and proximate properties of the polymers are presented in Table 1. The results showed that the WAC, swelling index, crude fiber and protein for *Irvingia* gum were significantly higher ($p < 0.05$) than that of *Cissus*. On the other hand, *Cissus* gum exhibited higher values of solubility, carbohydrate and ash. HPMC demonstrated higher solubility, the swelling and WAC could not be determined due to the free solubility in water and other proximate compositions were lower compared to the test polymers showing higher levels of purity. Water absorption capacity and swelling index are important functional parameters of polymers. The interaction between the gums with water showed that these polymers are hydrogels due to their ability to absorb and hold more than 50 times their weight of water as observed from the swelling index.

The formation of a stable gel by the polymers in aqueous media indicates the presence of tangles, cross-linkages or crystal-like regions within the polymer network.^{15,16} The suitability of gums as food or pharmaceutical excipients can be predicted by the dynamics of response when water moves in and out of hydrogels. The swollen state of hydrogels is a consequence of the balance between cohesive and hydration forces on the network of the polymer chains. Hydrophilic polymers, such as these gums, generally swell when water molecules interact with the polar groups mainly hydroxyl and carboxyl on the polymer backbone or side chains.¹⁶

The protein content of the polymers are ranked *Irvingia* < *Cissus* < HPMC and this could be attributed to their different botanical source. Similar hydrocolloids such as gelatin, Arabic gum, and mesquite are generally rich in protein and thus act as stabilizers. This is because they have sufficient hydrophobic groups which serve as bonding points as well as hydrophilic groups that reduce surface tension in a liquid-liquid or liquid-gas interface.^{17,18} The high content of carbohydrate found in the polymers respectively indicates the presence of sugar molecules.

Irvingia, *Cissus* and HPMC showed relatively low ash values in the order of HPMC < *Irvingia* < *Cissus*. Adulteration by sand or earth can be detected using the ash content which normally consists of inorganic mixtures of

Table 1. Functional and proximate properties of *Irvingia* and *Cissus* gum

Parameter	<i>Irvingia</i>	<i>Cissus</i>	HPMC
Solubility (%)	8.73 $\pm$ 0.04	15.73 $\pm$ 0.18	99.75 $\pm$ 0.03
WAC (%)	73.00 $\pm$ 2.11	50.60 $\pm$ 1.39	ND
Swelling capacity (%)	60.00 $\pm$ 2.34	53.00 $\pm$ 2.54	ND
Moisture content	9.55 $\pm$ 1.03	8.56 $\pm$ 2.09	4.87 $\pm$ 0.19
Crude fibre (%)	29.75 $\pm$ 3.11	9.80 $\pm$ 2.10	8.04 $\pm$ 2.17
Protein (%)	5.00 $\pm$ 0.98	3.00 $\pm$ 0.34	0.02 $\pm$ 0.00
Carbohydrate (%)	44.70 $\pm$ 1.21	74.25 $\pm$ 1.27	76.89 $\pm$ 6.81
Ash (%)	2.00 $\pm$ 0.02	3.00 $\pm$ 0.07	1.78 $\pm$ 0.01

ND = not determinable because of free solubility.

carbonates, phosphates, silicates and silica. Low ash values suggest the presence of higher organic than inorganic constituents, which exhibit high purity of the materials. The ash content of *Irvingia* was quite similar to that reported for *Chrysophyllum albidum* gum (2.0%) in a recent study¹⁸ while in *Cissus* it was higher. In addition, the ash content of *Irvingia* and *Cissus* was lower than that earlier reported for Xanthan gum.¹⁹ This implies, therefore, that the *Irvingia gabonensis* and *Cissus pulpunea* used in this study acquired low levels of contamination during gathering, extraction, processing and storage.

The results of elemental assessments for *Irvingia* and *Cissus* gums are presented in Table 2. They reveal the presence of calcium, magnesium, potassium, sodium, manganese, iron, copper and zinc, which are not harmful to the body, while heavy metals like cobalt, lead and cadmium, which may present toxicity, were absent. HPMC is not included, because official compendium has profiled it as GRAS.

Scanning electron micrographs (SEM) of the gums is presented in Fig. 1. *Irvingia* and *Cissus* gums showed characteristic irregularly-shaped particles with rough surfaces. Particle shape is one of the physical properties influencing powder packing and flow especially in tabletting procedures.²⁰

Table 2. Elemental composition of *Irvingia* and *Cissus* gum

Parameter	<i>Irvingia</i>	<i>Cissus</i>
Calcium	0.53	1.43
Magnesium	0.45	0.41
Potassium	0.94	1.14
Sodium	10.30	10.20
Manganese	1.56	0.51
Iron	0.38	0.77
Copper	0.33	0.53
Zinc	1.40	1.20
Cobalt	0.00	0.00
Lead	0.00	0.00
Nickel	0.21	0.36
Chromium	0.21	0.39
Cadmium	0.00	0.00

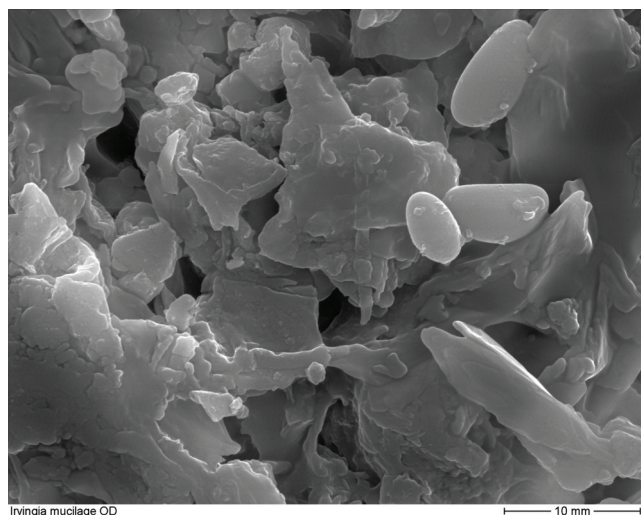


Fig. 1a. Scanning electron micrographs of *Irvingia* gum

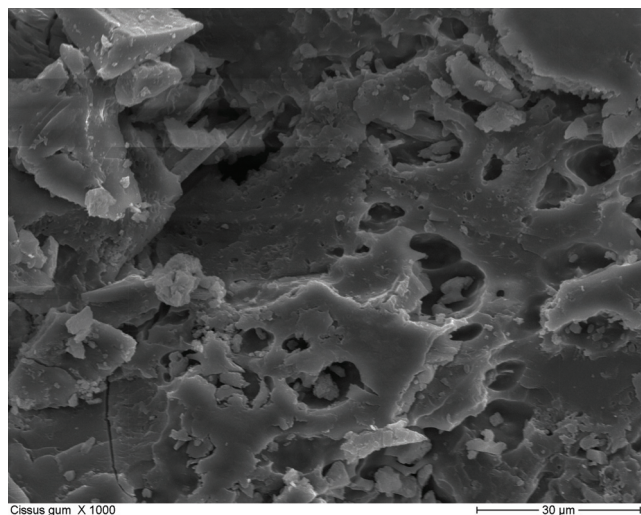


Fig. 1b. Scanning electron micrographs of *Cissus* gum (magnification $\times 1000$)

The viscosity profiles of the natural gums are presented in Fig. 2. *Irvingia*, *Cissus* and HPMC showed an increase in viscosity as concentration increases. This may be attributed to intermolecular friction exerted when layers of fluids attempt to slide over one another indicating that there is an increase in the internal resistance of the gum to flow as more molecules are available in the higher concentrations. *Cissus* gum generally showed a higher viscosity profile than *Irvingia*, probably due to structural differences or the presence of more hydrophilic groups. However, both gums showed high viscosity values compared to HPMC. The HPMC grade used in this study is E5 Premium LV; hence, the low viscosity. The addition of humectant increased the viscosity of all the polymers, though this effect was more pronounced with *Cissus* gum. Thus, the humectant appeared to modify the viscosity of the polymers to varying degrees.

The result of the moisture sorption of *Irvingia* and *Cissus* gums and HPMC at varying levels of relative humidity is given in Fig. 3. The moisture sorption profiles showed that the gums were moderately hygroscopic, as their moisture uptake was greater than 5% after storage below

60% RH, and less than 40 at 90% RH. There were also no significant differences ($p < 0.05$) in the moisture sorption of the 2 gums. HPMC showed slightly hygroscopic characteristics as the moisture uptake between 75 and 90% RH was less than 15%. In addition, the moisture sorption of *Cissus* and *Irvingia* gum was relatively high compared to HPMC, which showed the least sorption of moisture at relative humidity. Abramovic and Klofutar²¹ reported the case of low acyl gellan gum, which was found to absorb more water at high relative humidity, while at low relative humidity, it absorbed much less amounts of water.

Properties of films

Homogeneity and appearance of the films were examined by visual observation. The dried films of *Irvingia* and *Cissus* were opaque and flexible when handled and were easily removed from the cast plate. Films obtained using HPMC were transparent, glassy in appearance, flexible and easy to remove from the plates. Generally, films containing no humectant dried much faster than those containing humectant. Humectant has been reported as interfering with polymeric chain association, thus decreasing the rigidity of the network and producing a less ordered film structure.²²

The swelling properties of *Irvingia* and *Cissus* films are presented in Table 3. The results showed that the swelling index of the films reduced with the increase in concentration for both gums, though without a significant differ-

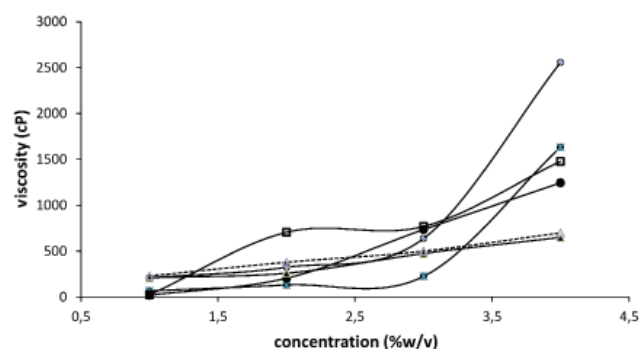


Fig. 2. Viscosity profiles of polymer dispersions (● – *Cissus*, ○ – *Cissus* + humectant, ■ – *Irvingia*, □ – *Irvingia* + humectant, ▲ – HPMC, △ – HPMC + humectant)

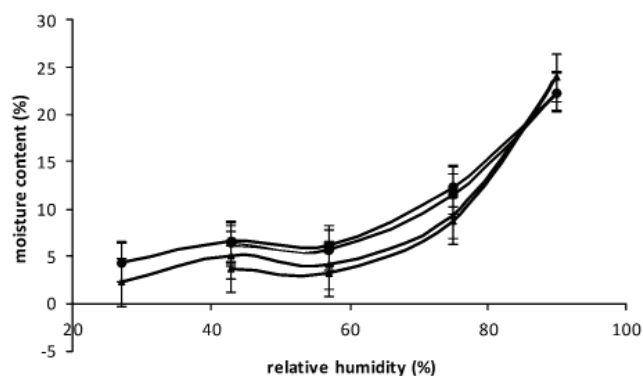


Fig. 3. Moisture sorption curve for gums of *Irvingia* (●), *Cissus* (■) and HPMC (▲)

Table 3. Swelling index of dried films at different pH (mean $\pm$ SD, n = 3)

Gum	Concentration (%w/v)	pH		
		4.75	6.0	7.4
<i>Irvingia</i>	2.0	5.87 $\pm$ 0.68	6.08 $\pm$ 0.75	6.52 $\pm$ 1.84
	3.0	5.63 $\pm$ 1.66	5.66 $\pm$ 0.55	6.18 $\pm$ 0.70
	4.0	5.44 $\pm$ 0.79	5.59 $\pm$ 0.18	6.01 $\pm$ 0.88
<i>Cissus</i>	2.0	10.08 $\pm$ 0.97	12.73 $\pm$ 1.90	10.76 $\pm$ 0.44
	3.0	6.97 $\pm$ 0.27	12.72 $\pm$ 1.00	10.55 $\pm$ 0.31
	4.0	6.49 $\pm$ 0.29	11.98 $\pm$ 1.09	10.32 $\pm$ 0.78
HPMC	2.0	ND	ND	ND
	3.0	ND	ND	ND
	4.0	ND	ND	ND

ND = not determinable because of free solubility.

ence. In addition, swelling increased with the increase in the pH of the medium for *Irvingia*, while it is reduced for *Cissus*. HPMC films absorbed the phosphate buffer rapidly within twenty minutes, causing a breakdown and erosion of the film. The reason for this may be the free solubility of HPMC causing the degradation of the polymer backbone, thus leading to film disintegration. Hence, HPMC dried film did not show any swelling compared to *Cissus* and *Irvingia* films. The swelling capacity provides evidence of the magnitude of interaction between polymer chains within the amorphous and crystalline domains.²³

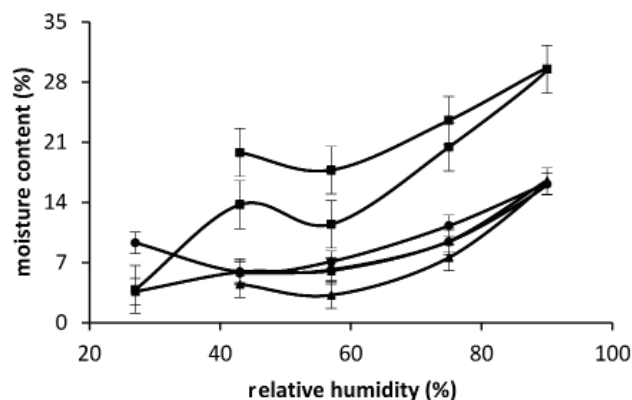
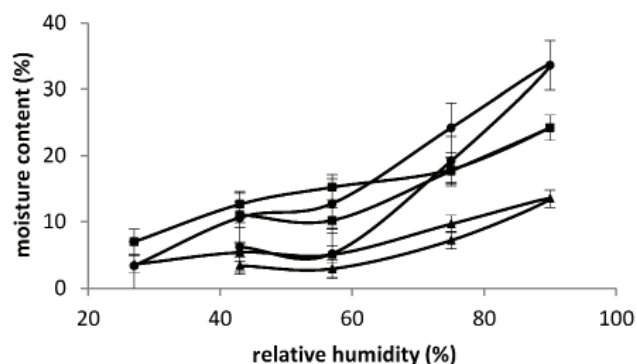
The results of the thickness of *Irvingia* and *Cissus* dried films are presented in Table 4. The thickness of films increased with the increase in the concentration of the gums, and films containing humectant were thicker, though without a significant difference ($p > 0.05$) for *Cissus*. The thickness of *Irvingia* films containing humectant

Table 4. Elemental composition of *Irvingia* and *Cissus* gum

Polymer	Concentration (%w/w)	Thickness (mm)
<i>Cissus</i>	2	0.110 $\pm$ 0.006
	3	0.120 $\pm$ 0.005
	4	0.130 $\pm$ 0.008
<i>Cissus</i> + humectant	2	0.120 $\pm$ 0.003
	3	0.120 $\pm$ 0.005
	4	0.130 $\pm$ 0.007
<i>Irvingia</i>	2	0.110 $\pm$ 0.001
	3	0.140 $\pm$ 0.015
	4	0.170 $\pm$ 0.021
<i>Irvingia</i> + humectant	2	0.120 $\pm$ 0.002
	3	0.220 $\pm$ 0.012
	4	0.240 $\pm$ 0.014
HPMC	2	0.065 $\pm$ 0.031
	3	0.069 $\pm$ 0.017
	4	0.096 $\pm$ 0.037
HPMC + humectant	2	0.074 $\pm$ 0.032
	3	0.102 $\pm$ 0.029
	4	0.107 $\pm$ 0.017

were, however, significantly higher ($p < 0.05$) than for those without. The humectant might have increased the thickness due to its capacity to increase the viscosity of the gums as shown in the viscosity profiles in Fig. 2.

Moisture sorption has been reported to be one of the most sensitive techniques for assessing the variation in the amorphous or crystalline content of polymers²⁴ as well as predicting some physicochemical and functional properties.²⁵ This is because the moisture uptake is predominantly due to the interaction of the water molecules with the amorphous part of the polymer network. The moisture sorption profiles of *Cissus* and *Irvingia* gums were quite similar as shown in Fig. 3. This could indicate a similarity in their polymer chain arrangement showing comparable amorphous domains.¹⁰ Figures 4 and 5 showed the moisture sorption profiles of films with and without humectant respectively. Generally, the moisture sorption increases with increase in relative humidity with the highest occurring at 90% RH for all films. At lower RH (43 and 57%), *Cissus* gum had significantly higher ($p < 0.05$) values than HPMC and *Irvingia* but at a higher RH (75 and 90%), *Irvingia* films showed higher moisture sorption. *Cissus* films containing humectant seems to adsorb a significantly higher ($p < 0.05$) amount of water than *Irvingia* and HPMC. This revealed that the effect of humectant modified the moisture sorption process in the films. Humectants are agents that help to conserve water in formulations, so as to avoid drying up.

**Fig. 4.** Moisture sorption curve of *Irvingia* (●), *Cissus* (■) and HPMC (▲) films containing humectant**Fig. 5.** Moisture sorption curve of *Irvingia* (●), *Cissus* (■) and HPMC (▲) films prepared without humectant

Tablet properties of uncoated and coated ibuprofen tablets

The mechanical and release properties of ibuprofen coated and uncoated tablets are presented in Table 5. The crushing strength (CS) for uncoated tablets was lower than that obtained for *Cissus*-coated and HPMC-coated tablets. This could be because of the decrease in porosity and subsequent formation of stronger bonds at closer inter-particle contact due to the concentration of the polymer gum.²⁶ The result also showed that the differences in the CS values of the uncoated and coated were significant ($p < 0.05$). Friability (F) test is a measure of the ability of tablets to withstand abrasion during shipping and handling. Conventional compressed tablets that lose less than 1% of their weight during the friability test are generally considered acceptable. Friability was significantly ($p < 0.05$) lower for *Cissus*-coated and HPMC-coated tablets than uncoated ones. This decrease may be attributed to the greater amount of particle-particle contact points which created more solid bonds, resulting in tablets with more resistance to fracture and abrasion, thus presenting higher crushing strength and lower friability. Generally, the mechanical properties of coated tablets as summarized by CS/Fr were significantly higher ($p < 0.05$) than that of uncoated showing improved properties.

The disintegration time (DT) of uncoated and *Cissus*-coated ibuprofen tablet is presented in Table 5. It was observed that the disintegration time of coated tablets was significantly higher ($p < 0.05$) than it was in the case of uncoated tablets. This could be a result of a decrease in tablet porosity or a reduction in the capillary microstructure of the polymer coat on the tablets.²⁷ Particle rearrangement, fragmentation and deformation may result in the closure of the intra and inter-granular pore spaces, thereby reducing the capillary microstructure of the tablets.²⁷ Consequently, water penetration into tablets would be retarded, leading to an increase in the disintegration. Tablet disintegration time for uncoated ibuprofen tablets was generally lower, probably due to the lack of particle re-arrangement, resulting in faster water penetration to facilitate disintegration. In addition, HPMC-coated tab-

Table 5. Tablet properties of uncoated and *Cissus*-coated Ibuprofen tablets

Parameters	Uncoated Ibuprofen tablets	3% w/v <i>Cissus</i> -coated Ibuprofen tablet	3% w/v HPMC-coated Ibuprofen tablet
Thickness (mm)	6.012 $\pm$ 0.010	6.030 $\pm$ 0.010	7.072 $\pm$ 0.073
Crushing strength (N)	53.220 $\pm$ 2.240	77.530 $\pm$ 3.910	76.673 $\pm$ 3.871
Friability	0.760 $\pm$ 0.010	0.200 $\pm$ 0.040	0.291 $\pm$ 0.043
CS/Fr	70.026	387.650	263.481
Disintegration time (min)	15.930 $\pm$ 0.940	68.930 $\pm$ 0.530	56.670 $\pm$ 3.192
t_{50}	12.032	19.103	32.592
t_{80}	26.035	36.105	48.543

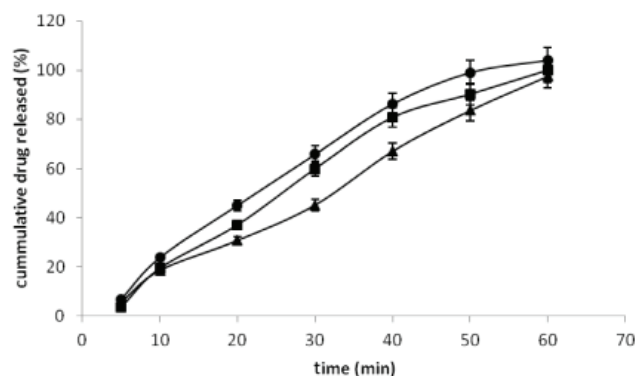


Fig. 6. Dissolution profiles for uncoated (●), *Cissus*-coated (■) and HPMC-coated (▲) Ibuprofen tablet

lets yielded a lower DT compared to *Cissus*-coated tablets. The BP stipulates 1-h for film-coated tablets while uncoated have 15 min. The HPMC-coated tablets disintegrated within 1 h showing a higher performance over the *Cissus*-coated tablets.

The dissolution profiles of ibuprofen drug from *Cissus*-coated, HPMC-coated tablets and uncoated tablets are shown in Fig. 6 and the values of t_{50} and t_{80} (time required for 50 and 80% of ibuprofen to be released respectively) are included. The dissolution profiles showed that 80% of the drug was released in 36 min and 50% of the drug released at 19 min for *Cissus*-coated tablets, while uncoated ibuprofen tablet, gave 80% of drug release at 26 min and 50% of drug release at 12 min. HPMC-coated tablets yielded 32.59 and 48.5 for 50 and 80% drug release. Thus, tablet coated using *Cissus* gum and HPMC had higher dissolution times than uncoated tablets, showing that the coating can be used to control the release of ibuprofen.

Conclusion

The proximate, functional and elemental properties of *Irvingia* and *Cissus* gums in this study have demonstrated their potential as polymers for pharmaceutical use. The mechanical properties of *Cissus*-coated tablets improved, while there was a delay in drug release, revealing that the film provided effective coating.

References

- Kulkarni AP, Shaikh YR, Dehghan GR. Application of neem gum for aqueous film coating of ciprofloxacin tablets. *Int J Appl Res Nat Prod.* 2013;16(3):1–5.
- Ogaji IJ, Nep EI, Audu-Peter JD. Advances in natural polymers as pharmaceutical excipients. *Pharm Anal Acta.* 2012;3:146–151.
- Ibrahim H, Rai PP, Bangudu AB. Pharmacognostic studies of the Stem Bark of *Cissus polypnea* Guill & Perr. *Glimp Plant Res.* 1993;1:175–180.
- Iwe MO, Attah MA. Functional properties of the active ingredients of *Cissus Pulpnea* Guill. Perr. Books Ltd. Ibadan Nig. 2nd ed. 1993;289–292.
- Joseph JK. Physico-chemical attributes of Wild Mango (*Irvingia gabonensis*) Seeds. *Bioresource Technology.* 1995;53:179–181.

6. Ladipo DO, Fondoun JM, Ganga N. Domestication of the Bush Mango (*Irvingia* Spp). Some exploitable intraspecific variations in west and central Africa. In: Domestication and International Conference Held In Nairobi, Kenya. Non-Wood Forest Products. Food and Agriculture Organisation (E.A.O). Rome, Italy 1996;9:193–205.
7. Agbor LON. Marketing trends and potentials for *Irvingia gabonensis* products in Nigeria. Proc. ICRAF-ILTA Conf. *Irvingia gabonensis*, May 3–5, Ibadan, Nigeria, 1994.
8. Odeku OA, Patani B. Evaluation of dika nut mucilage (*Irvingia gabonensis*) as a binding agent in metronidazole tablet formulation. *Pharm Dev Technol.* 2005;10:439–446.
9. Isimi CY, Kunle OO, Bangudu AB. Some emulsifying and suspending properties of the mucilage extracted from kernels of *Irvingia gabonensis*. *Boll Chim Farm.* 2000;139:199–204.
10. Odeku OA, Lamprecht A, Okunlola A. Characterization and evaluation of four natural gums as polymers in formulations of diclofenac sodium microbeads. *Int J Biol Macromol.* 2013;58:113–120.
11. Odeku OA, Itiola OA. Evaluation of the effect of Khaya gum on the mechanical and release properties of paracetamol tablets. *Drug Dev Ind Pharm.* 2003;29(3):311–320.
12. Association of Official Analytical Chemists (A.O.A.C) Official Methods of Analysis, Centennial Ed. 14, Washington D.C USA. 1984, ISBN 13: 9780935584240.
13. Kaur M, Oberoi DPS, Sogi DS, Gill BS. Physicochemical, morphological and pasting properties of acid treated starches from different botanical sources. *J Food Sci Technol.* 2011;48(4):460–465.
14. Lin YC, Chen X. Moisture sorption–desorption–resorption characteristics and behaviour of the epoxy system. *Polym.* 2005;46:11994–12003.
15. Kosmulski M, Gustafsson MJ, Rosenholm JB. Ion specificity and viscosity of rutile dispersions. *Col Polym Sci.* 1999;277(6):550–556.
16. Galindo-Rodríguez SA, Puel F, Briançon S, Allémann E, Doelker E, Fessi H. Comparative scale-up of three methods for producing ibuprofen-loaded nanoparticles. *Eur J Pharm Sci.* 2005;25:357–367.
17. Lopez VO, Garcia MA, Zaritzky EN. Film forming capacity of chemically modified corn starches. *Carb Polym.* 2007;73:573–581.
18. Ajala TO, AkinAjani OD, Ihuoma-Chidi C, Odeku OA. *Chrysophyllum albidum* mucilage as a binding agent in paracetamol tablet formulations. *J Pharm Inves.* 2016; 46(6):565–573.
19. Sciarini LS, Maldonado F, Ribotta PD, Pérez GT, León AE. Chemical composition and functional properties of *Gleditsia triacanthos* gum. *Food Hydrocol.* 2009;23:306–313.
20. Okafor IS, Chukwu A, Udeala OK. Some physical properties of *Grewia* gum. *Nig J Polym Sci Tech.* 2001;2(1):76–83.
21. Abramovic H, Klofutar C. Water adsorption isotherms of some gelatin gum samples. *J Food Eng.* 2006;77(3):514–520.
22. Sothornvit R, Krochta JM. Plasticizers in edible films and coatings, in Innovations. In: J. H. Han (Ed.), Innovations in food packaging Amsterdam, The Netherlands: Elsevier. 2005:403–428.
23. Hover R. Composition, molecular structure and physicochemical properties of tuber and root starches: a review. *Carbo Polym.* 2001;4:29–35.
24. Manek RV, et al. Physical, thermal and sorption profile of starch obtained from *Tacca leontopetaloides*. *Starch–Starke* 2005;57(2):55–61.
25. Bravo-Osuna I, Ferrero C, Jiménez-Castellanos MR. Water sorption-desorption behaviour of methyl methacrylate-starch copolymers: Effect of hydrophobic graft and drying method. *Eur J Pharm Biopharm.* 2005;59:537–548.
26. Beery KE, Ladisch MR. Chemistry and properties of starch-based dessicants. *Enzym Microbial Tech.* 2001;28:573–581.
27. Luangtanan-Anan M, Fell JT. Bonding mechanisms in tableting. *Inter J Pharm.* 1990;60:197–202.

Native and microwave-modified *Terminalia mantaly* gums as sustained-release and bioadhesive excipients in naproxen matrix tablet formulations

Michael Ayodele Odeniyi^{1,A–F}, Babatunde Mukhtar Oyedokun^{1,B–D}, Oluyemisi Adebawale Bamiro^{2,C–E}

¹ Department of Pharmaceutics and Industrial Pharmacy, University of Ibadan, Ibadan, Nigeria

² Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Olabisi Onabanjo University, Sagamu, Nigeria

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;

D – writing the article; E – critical revision of the article; F – final approval of article

Polymers in Medicine, ISSN 0370-0747 (print), ISSN 2451-2699 (online)

Polim Med. 2017;47(1):35–42

Address for correspondence

Michael Ayodele Odeniyi

E-mail: deleodeniya@gmail.com

Funding sources

none declared

Conflict of interest

none declared

Received on April 11, 2017

Revised on July 06, 2017

Accepted on July 24, 2017

Abstract

Background. Hydrophilic polymers provide a means of sustaining drug delivery. Native gums may be limited in function, but modification may improve their activity.

Objectives. The aim of the study was to evaluate native and modified forms of *Terminalia mantaly* gum for their sustained-release and bioadhesive properties.

Material and methods. The native gum (NTM) was modified by microwave irradiation for 20 seconds (MTM20) and 60 seconds (MTM60) and characterized using microscopy, Fourier transform infrared spectroscopy (FTIR) and packing properties. The effects of the thermally induced molecular reorientation were determined. Tablet formulations of naproxen were produced by direct compression. The mechanical, bioadhesive and release properties of the formulations were determined.

Results. Irradiation of NTM improved the gum's flow properties, resulting in Carr's Index and Hausner's ratios lower than 16% and 1.25, respectively. Swelling studies showed that MTM20 and MTM60 had lower water absorption capacity and swelling index values, while packing properties improved upon irradiation, as depicted by lower tapped density values. FTIR spectra of samples showed that the irradiated gums were distinct from the native gums and did not interact with naproxen sodium. The gum's mechanical properties improved with MTM20 and MTM60 and sustained-release action of up to 12 h was obtained.

Conclusions. Inclusion of hydroxypropyl methylcellulose (HPMC) in the tablet formulations proved critical for bioadhesion. Microwave irradiation of native *Terminalia mantaly* gum improved the flow, mechanical and sustained-release properties of Naproxen tablets, and the addition of HPMC increased bioadhesion properties. The tablet properties of the native gum were significantly improved after 20 s of microwave irradiation.

Key words: sustained-release, microwave irradiation, bioadhesion, Terminalia gum, naproxen sodium

DOI

10.17219/pim/76058

Copyright

© 2017 by Wrocław Medical University

This is an article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

Polymers are becoming increasingly important in the field of drug delivery. The pharmaceutical applications of polymers range from their use as binders in tablets to viscosity- and flow-controlling agents in liquids, suspensions and emulsions. Polymers can also be used as film coatings, to disguise the unpleasant taste of a drug, to enhance drug stability and to modify drug release characteristics.¹ Plant-based polymers have been studied for their applications in different pharmaceutical dosage forms, such as matrix-controlled systems, buccal films, microspheres, nanoparticles, viscous liquid formulations like ophthalmic solutions, suspensions and implants, and their applicability and efficacy has been proven. Polymers have also been utilized as viscosity enhancers, stabilizers, disintegrants, solubilizers, emulsifiers, suspending agents, gelling agents, bioadhesives and binders.^{2,3}

The goal of designing a sustained delivery system is to reduce the frequency of dosing while maintaining uniform drug delivery. Generally, sustained-release dosage forms provide better control of drug levels in plasma, reduce dosage frequency and increase therapeutic efficacy through steady drug delivery.⁴

The bioadhesive property of certain polymeric systems has found relevance in drug delivery as a means of prolonging contact time in the various mucosal routes of drug administration. The ability to maintain a delivery system at a particular location for an extended period of time has great appeal for local action as well as systemic drug bioavailability.⁵ Natural gums have been found to be bio-compatible and non-irritating, and they possess excellent binding and bioadhesive properties.⁶ However, there may be a need to modify these gums in order to achieve the desired formulation effects. Microwave irradiation has provided a convenient means of modifying polymers by reducing processing time.^{7,8} While a few gums have been modified by grafting functional groups through microwave irradiation,^{9,10} the present work seeks to determine the effect of duration of irradiation on the mechanical properties, bioadhesion characteristics and sustained-release properties of a new plant gum from the *Terminalia mantaly* tree in a directly compressed matrix tablet.

Terminalia is a genus of large trees of the flowering plant family *Combretaceae*, comprising around 100 species distributed in tropical regions of the world. Naproxen, the candidate drug, is a non-steroidal anti-inflammatory drug of the propionic acid class that relieves pain, fever, swelling and stiffness. A sustained-release form will be useful in treating rheumatoid arthritis, osteoarthritis and ankylosing spondylitis.

Material and methods

The materials used include naproxen sodium, which was received as a gift from Swiss Pharma Nigeria Ltd.

(Lagos, Nigeria); *Terminalia* gum, extracted from a *Terminalia mantaly* tree trunk located at the Botanical Gardens, University of Ibadan, Nigeria; hydroxypropyl methyl cellulose (HPMC) (Colorcon Asia Limited, Mumbai, India); microcrystalline cellulose (Aqualon Group, Hercules Inc., Wilmington, DE, USA); Aerosil®200 (Evonik Degussa Corp., Essen Germany); magnesium stearate (R&M Chemicals, Essex, UK). Other reagents were of analytical grade.

Preparation of the gum

The plant sample was initially identified and authenticated at the University of Ibadan Herbarium (voucher number UIH-22529). The gum was purified using previously described methods.^{11,12} Briefly, the exudate was hydrated in a 0.5 : 95.5 (v/v) CHCl₃/water mixture for five days with intermittent stirring; extraneous materials were removed by straining through a muslin cloth. Absolute ethanol was used to precipitate the gum from the solution. The precipitated gum was filtered, washed with diethyl ether, and then dried in a hot air oven at 40°C for 18 hours. The resultant pure gum was pulverized using a laboratory blender, sieved, and stored in an airtight container.

Modification of the *Terminalia* gum by microwave irradiation

A 40 g quantity of the native gum was made into a slurry with distilled water. The slurry was dried in divided quantities on square ceramic tiles with the aid of a 2450 MHz microwave unit (Model R-218L, Sharp Corp., Sakai, Japan) which released 800 watts of heat energy for 20 seconds (MTM20) and 60 seconds (MTM60). The microwave irradiated samples were subsequently dried in an oven set at 60°C for 24 hours. The dried gums were scraped off the tiles and milled using a laboratory blender. The powder was passed through a sieve (mesh size 120) to obtain uniform sized particles.

Interactions between *Terminalia* gum and naproxen sodium

Fourier transform infrared spectroscopy (FTIR) spectra were obtained for the pure gum and naproxen sodium samples, and for 1 : 1 mixtures of each gum sample and naproxen sodium, in order to determine if interactions occurred between the gums and the active ingredient. Each sample powder (1.0 mg) was finely ground to about 2.0 µm in size and mixed with approximately 100 mg of dry potassium bromide (KBr). Each sample was analyzed in a Spectrum RX-1 Fourier Transform Infrared Spectrometer (Perkin Elmer Ltd., Beaconsfield, UK).

Formulation design for naproxen sodium matrix tablets

The formulation design utilized in the manufacture of the naproxen matrix tablets used in this study is given in Table 1. Twelve batches (F1–F12) of naproxen tablets were prepared. F1–F4 contain NTM, F5–F8 contain MTM20, while F9–F12 contain MTM60.

Preparation of matrix tablets

Matrix tablets were produced by weighing, screening and mixing the excipients through a 40-mesh sieve, then adding the active ingredient and mixing thoroughly. The bulk density and tapped density of the powder blend was determined with graduated cylinders according to USP guidelines. The powders were compressed using a tabletting machine (Manesty Machines Ltd., Liverpool, England) fitted with round concave-faced 10 mm diameter punches and dies. The compression force was 1 metric ton.

Evaluation of the tablets

Twenty tablets were randomly selected from each batch and the average weight of the tablets was determined. Tablet thickness was measured for all tablets in each batch using a micrometer screw gauge. The crushing strength of the tablets was determined at room temperature by diametral compression¹³ using a hardness tester (Model EH 01, Copley Scientific, Mumbai, India). The results were taken only from tablets which split cleanly into two halves without any sign of lamination. The determinations were done in triplicate and the mean was calculated as the crushing strength.

Determination of ex vivo mucoadhesive strength

In determining the ex vivo mucoadhesive strength of the formulations, a segment of the intestine of a freshly sacrificed cow was fixed, using an elastic rubber band, onto a stainless steel cylinder with the basolateral side facing the cylinder.¹⁴ Tablets containing different concentrations

of the gum were pressed on the apical side and the cylinder was placed into 500 mL of a pH 6.8 buffer medium. The rotation speed was set at 50 rpm. The elapsed time for the tablet to detach from the mucosa was observed and recorded.¹⁵

Drug release

The in vitro drug dissolution study was carried out in 900 mL of 0.1 M HCl at $37.0 \pm 0.5^\circ\text{C}$ for the first hour and in pH 6.8 phosphate buffer for 11 h (mimicking drug release within the intestine), using the USP basket method at a stirring speed of 100 rpm. Samples were withdrawn at predetermined intervals and immediately replaced with an equal volume of fresh dissolution medium. The samples were filtered using a $0.45\ \mu\text{m}$ membrane filter and the amount of drug released was determined using a UV spectrophotometer (Hitachi U2000, Tokyo, Japan) at 231 nm.

Mechanism of drug release

The mechanism of drug release from the matrix tablets was determined by fitting data into different release models as previously described.¹⁶ Further, the mean dissolution time (MDT) – a more accurate method for assessing drug release rate than $t_x\%$ – was obtained by fitting the dissolution data into the equation:

$$\text{MDT} = (n/n + 1)k^{-1/n} \quad (1),$$

where n is the release exponent and k is release rate constant.¹⁷ A higher MDT value indicates that the polymer has a higher drug retaining ability.¹⁸ The drug retarding efficiency and release rate can be obtained from the equation. Further, the Akaike criterion index (AIC) was used to determine the most suitable model using DDSolver, an add-in program for Microsoft Excel.¹⁹

Statistical analysis

The statistical analysis was carried out using Students' t-test and ANOVA, with $p \leq 0.05$ considered significant.

Table 1. Formulation design for naproxen sodium matrix tablets

[illegible]

Results and discussion

Physicochemical properties of the gum samples

The presence of any interaction between the drug and excipient was evaluated using the FTIR spectroscopy method. The FTIR spectra of the pure drug, pure gum and gum-drug physical mixtures were measured. The major peaks observed for the pure samples remained discernible (Fig. 1) in the gum-drug mixtures. This means that little or no interaction occurred between the polymers and naproxen sodium.

The micromeritic properties of the formulations are given in Table 2. There were no significant differences in the bulk and tapped densities of the formulations. The angle of repose obtained for the formulation blends was above 40°, indicating uneven flow.

The flow properties of a powder are essential for determining its suitability as direct compression excipients.²⁰ Flow properties were determined by Hausner's ratio, Carr's index and the angle of repose (Table 3). The results obtained for Carr's index were 35% for NTM, 10.67% for MTM20 and 13.79% for MTM60. The greatly reduced values (less than 16%) for the microwaved gums indicated a significant improvement in flow properties compared to the native gum, which tended towards cohesiveness at 35%. The Hausner's ratios were 1.54 (NTM), 1.12 (MTM20) and 1.16 (MTM60). Both values for the irradiated gums were less than 1.25, indicating good flow. The native gum, however, had a value greater than 1.25, sug-

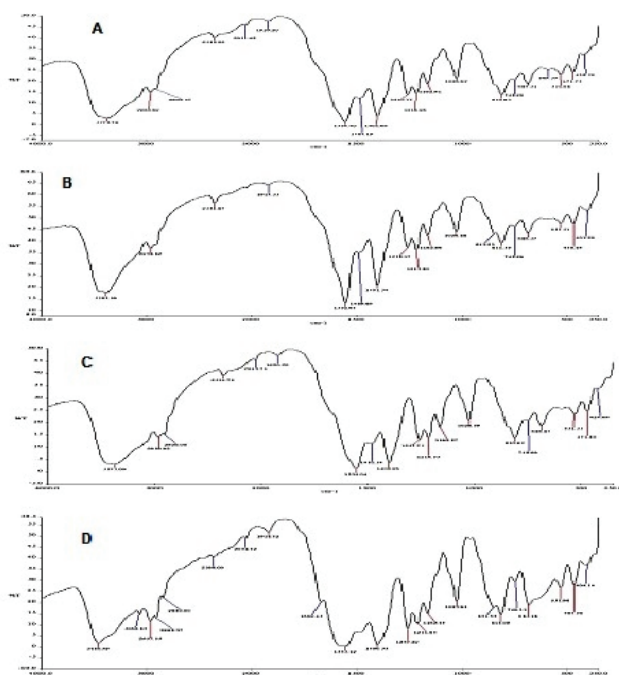


Fig. 1. FTIR spectra of 1 : 1 physical mixtures of (A) native gum with naproxen; (B) microwaved gum with naproxen (MTM20); (C) microwaved gum with naproxen (MTM60); and (D) pure naproxen sodium

Table 2. Density measurements and flow properties of formulation blends

Blends	Bulk density (g/cm ³)	Tapped density (g/cm ³)	Angle of repose (°)
F1	0.168	0.200	55.4
F2	0.159	0.199	53.6
F3	0.177	0.193	52.9
F4	0.182	0.205	51.5
F5	0.177	0.201	50.8
F6	0.174	0.207	51.5
F7	0.176	0.224	53.6
F8	0.171	0.199	52.2
F9	0.159	0.187	54.2
F10	0.171	0.199	55.4
F11	0.163	0.199	54.2
F12	0.161	0.199	54.2

gesting that irradiation of native *Terminalia mantaly* gum significantly improves its flow. There were no significant differences in the angles of repose observed for *Terminalia mantaly* gum samples. The ranking was in the following order: MTM20 (58.9°) > MTM60 (58.74°) > NTM (57.8°). The values were observed to be slightly higher for the irradiated gums. However, all values were higher than 40° but lower than 60°, indicating uneven flow.

Particle size influences the flow properties of powders; the greater the particle size, the greater the ease of flow. There was a progressive increase in the particle size of the gums as the duration of exposure to microwave irradiation increased. The increase in size is likely due to swelling of the gum particles upon the addition of water during mucilage formation, prior to irradiation. The average particle diameter of MTM60 (1316.22 µm) was five times that of the average NTM particle diameter (263.10 µm), while the average particle diameter of MTM20 (696.78 µm) was more than twice the average particle diameter of NTM (Table 3). Although the angles of repose observed for the gum samples showed only slight differences, the increased particle sizes of the microwaved gums indicates better flow. The ranking of the ease of flow was MTM60 > MTM20 > NTM.

Table 3. Physicochemical properties of *Terminalia mantaly* gum

Parameters	Native gum	Microwaved gum (20 s)	Microwaved gum (60 s)
Particle diameter (µm)	263.10	696.78	1316.22
Angle of repose (°)	57.80	58.09	58.74
Particle density (g/cm ³)	1.32	1.22	1.29
Bulk density (g/cm ³)	0.090	0.092	0.10
Tapped density (g/cm ³)	0.139	0.103	0.116
Hausner's ratio	1.54	1.12	1.16
Carr's index	35	10.67	13.79
Swelling index	8.4	7.2	7.6
Water absorption capacity	10.71	9.20	9.61
pH	7.31	5.37	6.22

Evaluation of naproxen tablet formulations

All the matrix tablets were evaluated for their physical, mechanical and release properties. The tablets produced from the powder blends had uniform thickness, low friability and a high degree of weight uniformity (Table 4). It is important to ensure that tablets in each batch of formulation fall within the appropriate size range, as this will affect chemical content.²¹ The British Pharmacopoeia states that for tablets having mean weights of greater than 250 mg, no more than two tablets are permitted to deviate from the mean by greater than $\pm 5\%$ and no tablet should deviate by more than $\pm 10\%$. The results obtained (Table 4) shows that the highest deviation observed was 0.02%. Thus, all the matrix tablet batches were satisfactory in this regard. Generally, excessive weight variation is attributable to poor granule flow during compression, improper die filling or the presence of air in the powder/granule bed.²¹

Crushing strength provides a measure of tablet strength, while friability is a measure of tablet weakness.²² In this study, the highest crushing strength was observed with MTM60 (40.41 N, tablet F9), followed by MTM20 (35.40 N, tablet F6) and NTM (21.43 N, tablet F2). Matrix tablets F9, F6 and F2 contained 40 mg (10%) of the gum samples. Further, comparison can be made between the crushing strengths of matrix tablet batches containing the same concentrations of the gum samples. Tablets F1 (13.44 N), F5 (16.23 N) and F9 (23.19 N) contained 20 mg (5%) of NTM, MTM20 and MTM60, respectively. It is also noteworthy that tablets F1, F5 and F9 contain 10 mg, 20 mg and 30 mg of HPMC, respectively (Table 4).

Table 4 also clearly shows that crushing strength increased as the gum and HPMC concentrations increased, resulting in the ranking MTM60 > MTM 20 > NTM. It can also be seen that there was a marked reduction in the crushing strengths of matrix tablets F4, F8 and F12, which shows that the exclusion of HPMC resulted in tablets with low mechanical strength.

Friability is a disruptive force used to evaluate the ability of tablets to withstand chipping and breakage during use. A maximum weight loss of 1% is usually acceptable for tablets.²³ Table 3 shows friability results ranging from 1.87% (tablet F11) to 4.09% (tablet F8). The failure to meet the specifications of the friability test may be due to low binder concentration, resulting in loose interparticulate bonding, or the use of low compression pressure in the tablet machine.²¹ Thus, the binder concentrations and compression pressure of 0.5 metric tons used for this study might be sub-optimal for formulating tablets which satisfy specifications for friability.

The crushing strength/friability ratio (CSFR) provides a measure of tablet strength and weakness. CSFR has been described as a useful index for tablet quality.¹² Also, Bamiro et al. reported that the higher the value of this index, the stronger the tablet.²⁴ The results obtained (Table 4) generally showed an increase in CSFR and hence, tablet strength, as binder and HPMC concentrations increased. Higher CSFR values were noted for tablets F9, F10 and F11, which all contain MTM60. The ranking was in the order MTM60 > MTM20 > NTM. The lowest values were obtained for matrix tablets F4, F8 and F12, none of which contain HPMC. These findings are consistent with deductions made from the crushing strength results, which suggests that while binding activity is greater with MTM60 and MTM20 relative to NTM, the inclusion of HPMC increases tablet strength.

Release studies on naproxen tablet formulations

Tablet disintegration has been described as the net outcome of adhesive and disintegrating forces that are activated when a tablet is subjected to an aqueous environment. Disintegration time is usually a necessary step for dissolution, and it could be the rate-determining step in the process of drug absorption.²⁵ This is because a drug entity must be present in solution before it can be absorbed, and this requires an initial disintegration process.

Table 4. Mechanical properties of naproxen matrix tablets

Tablet batches	Weight uniformity (mg)	Thickness (mm)	Crushing strength (N)	Friability (%)	Crushing strength/Friability ratio (CSFR)
F1	0.39 $\pm$ 0.01	3.59 $\pm$ 0.12	13.44 $\pm$ 3.74	2.22 $\pm$ 0.01	6.05
F2	0.41 $\pm$ 0.01	3.76 $\pm$ 0.18	21.43 $\pm$ 11.30	2.70 $\pm$ 0.04	7.94
F3	0.40 $\pm$ 0.02	3.75 $\pm$ 0.26	16.18 $\pm$ 0.00	2.24 $\pm$ 0.11	7.22
F4	0.39 $\pm$ 0.02	3.60 $\pm$ 0.17	15.84 $\pm$ 0.00	2.59 $\pm$ 0.02	6.12
F5	0.41 $\pm$ 0.02	3.71 $\pm$ 0.17	16.23 $\pm$ 0.55	1.95 $\pm$ 0.01	8.32
F6	0.41 $\pm$ 0.01	3.76 $\pm$ 0.13	35.40 $\pm$ 0.07	2.25 $\pm$ 0.05	15.73
F7	0.39 $\pm$ 0.01	3.64 $\pm$ 0.12	27.78 $\pm$ 14.59	3.16 $\pm$ 0.13	8.79
F8	0.40 $\pm$ 0.01	3.68 $\pm$ 0.13	14.96 $\pm$ 4.86	4.09 $\pm$ 0.02	3.66
F9	0.40 $\pm$ 0.01	3.72 $\pm$ 0.12	23.19 $\pm$ 5.06	2.81 $\pm$ 0.03	8.25
F10	0.40 $\pm$ 0.01	3.72 $\pm$ 0.11	40.41 $\pm$ 2.49	2.16 $\pm$ 0.01	18.71
F11	0.41 $\pm$ 0.01	3.65 $\pm$ 0.07	29.68 $\pm$ 0.00	1.87 $\pm$ 0.04	12.66
F12	0.41 $\pm$ 0.01	3.67 $\pm$ 0.08	16.77 $\pm$ 8.11	2.31 $\pm$ 0.12	7.26

Among the tablets incorporating HPMC, disintegration time decreased as polymer concentration increased, regardless of increases in HPMC concentration. Thus, for tablet batches containing NTM, MTM20 and MTM60, the longest disintegration time was observed at the lowest polymer concentration of 5% (20 mg). This suggests that as polymer concentration increased, the swelling and erosion rates of the tablets increased.

Generally, the matrix tablets containing NTM had the longest disintegration times – not less than 3 hours – and thus appear to be most suitable for sustained-release action. The ranking was NTM > MTM20 > MTM60. For the tablets containing no HPMC, the ranking was MTM20 > NTM > MTM60. Hence, in the absence of HPMC, MTM20 had the best sustained-release action.

The crushing strength-friability/disintegration time ratio (CSFR/DT) is a good index of tablet quality because it measures tablet strength (CS) and weakness (friability), which are indicators of the bond strength; and simultaneously evaluates any negative effect of these parameters on disintegration time, which is an indicator of bond disruption. A high CSFR/DT value indicates a good balance between binding and disintegration properties. Generally, the higher the CSFR/DT values, the better the disintegration of the tablet.²⁶

Matrix tablets F2, F6 and F10, which contained 40 mg of gum polymer, were found to possess the highest CSFR/DT values. CSFR/DT values also increased as the proportions of HPMC increased (Table 5). Matrix tablets F4, F8 and F12, containing HPMC, had the lowest CSFR/DT values. The ranking was generally MTM20 > MTM60 > NTM.

Bioadhesion times were studied for all the matrix tablets. It was generally observed that matrix tablets F4, F8 and F12 (which did not contain HPMC) had the poorest adhesion strength as shown by their short bioadhesion times (Table 5). Thus, the inclusion of HPMC improved bioadhesion. Among the matrix tablets containing

Table 5. Disintegration and bioadhesion times for naproxen matrix tablets (mean $\pm$ sd, n = 3)

Formulations	Disintegration time (hr)	Crushing strength – friability/disintegration time ratio (CSFR/DT)	Bioadhesion time (mins)
F1	3.53 $\pm$ 0.51	1.71	13.05 $\pm$ 1.05
F2	3.42 $\pm$ 0.35	2.32	49.22 $\pm$ 3.11
F3	3.08 $\pm$ 0.09	2.34	16.14 $\pm$ 2.20
F4	2.58 $\pm$ 0.17	2.37	5.06 $\pm$ 0.09
F5	3.17 $\pm$ 0.05	2.62	20.01 $\pm$ 2.01
F6	2.13 $\pm$ 0.42	7.30	21.15 $\pm$ 4.50
F7	2.00 $\pm$ 0.16	4.40	16.18 $\pm$ 1.01
F8	2.70 $\pm$ 0.11	1.36	8.05 $\pm$ 2.08
F9	2.53 $\pm$ 0.28	3.26	11.00 $\pm$ 1.15
F10	1.27 $\pm$ 0.16	14.73	12.15 $\pm$ 2.25
F11	2.18 $\pm$ 0.63	5.81	18.58 $\pm$ 1.06
F12	2.33 $\pm$ 0.44	3.12	4.06 $\pm$ 0.05

Table 6. Model independent dissolution parameters for naproxen matrix tablets

Formulations	Disintegration time (hr)	Crushing strength – friability/disintegration time ratio (CSFR/DT)	Bioadhesion time (mins)
F1	3.53 $\pm$ 0.51	1.71	13.05 $\pm$ 1.05
F2	3.42 $\pm$ 0.35	2.32	49.22 $\pm$ 3.11
F3	3.08 $\pm$ 0.09	2.34	16.14 $\pm$ 2.20
F4	2.58 $\pm$ 0.17	2.37	5.06 $\pm$ 0.09
F5	3.17 $\pm$ 0.05	2.62	20.01 $\pm$ 2.01
F6	2.13 $\pm$ 0.42	7.30	21.15 $\pm$ 4.50
F7	2.00 $\pm$ 0.16	4.40	16.18 $\pm$ 1.01
F8	2.70 $\pm$ 0.11	1.36	8.05 $\pm$ 2.08
F9	2.53 $\pm$ 0.28	3.26	11.00 $\pm$ 1.15
F10	1.27 $\pm$ 0.16	14.73	12.15 $\pm$ 2.25
F11	2.18 $\pm$ 0.63	5.81	18.58 $\pm$ 1.06
F12	2.33 $\pm$ 0.44	3.12	4.06 $\pm$ 0.05

HPMC, the longest bioadhesion time occurred with tablet F2. The proportion of NTM and HPMC in tablet F2 appear to be the optimum for achieving longer bioadhesion (assuming uniform attachment force). The shortest bioadhesion time was 11 min (tablet F9).

It was observed that as polymer concentration increased (from 5 to 10%) across F1–F2, F5–F6 and F9–F10, bioadhesion times increased correspondingly. However, a further increase in polymer concentration to 15% in tablets F3, F7 and F11 led to shorter bioadhesion times than observed at 10%, which suggests that bioadhesion is reduced in the presence of HPMC beyond a polymer concentration of 10%. Generally, the tablets containing MTM20 showed longer bioadhesion times relative to tablets containing MTM60. The ranking was NTM > MTM20 > MTM60.

Dissolution parameters were obtained for all the matrix tablet batches in 0.1 M HCl and pH 6.8 phosphate buffer solutions. The tablets were first evaluated in the 0.1 M HCl buffer for the first hour before being immersed in the pH 6.8 phosphate buffer for 11 subsequent hours. The percentage drug release in HCL buffer was negligible for all the matrix tablet batches, with the major part of the drug being released in the phosphate buffer. This might suggest suitability for targeted delivery within the intestinal lumen.

As noted above, matrix tablets F1–F4 contained native *Terminalia* gum, while tablets F5–F8 and F9–F12 contained gum irradiated for 20 s and 60 s, respectively. Matrix tablets containing native gum (F1–F4) generally had the lowest percentage of drug release. The release rate was found to increase with irradiation time for the microwave-modified gum samples. This can be explained by the observed increase in particle diameter and surface area of the gum particles after exposure to irradiation (Table 3), resulting in better solvent uptake and swelling.

Generally lower percentage drug release rates were observed for matrix tablets which did not contain HPMC: F4,

Table 7. In vitro release kinetics for naproxen matrix tablet formulations

Formulation code	Zero-order		First-order		Higuchi		Hixson-Crowell		Korsmeyer-Peppas			AIC
	r^2	k_0	r^2	k_1	r^2	K_H	r^2	K_{HC}	N	r^2	K	
F1	0.965	0.117	0.947	0.002	0.831	2.139	0.955	0.000	1.132	0.966	0.053	78.01
F2	0.970	0.115	0.952	0.001	0.838	2.101	0.960	0.000	1.117	0.970	0.057	75.23
F3	0.967	0.111	0.951	0.001	0.839	2.043	0.958	0.000	1.117	0.968	0.056	75.03
F4	0.958	0.065	0.964	0.001	0.901	1.203	0.963	0.000	0.841	0.963	0.166	60.19
F5	0.957	0.127	0.970	0.002	0.901	2.374	0.968	0.001	0.828	0.963	0.353	79.74
F6	0.962	0.134	0.970	0.002	0.910	2.504	0.970	0.000	0.823	0.969	0.383	78.17
F7	0.967	0.099	0.964	0.001	0.867	1.821	0.966	0.000	0.978	0.964	0.112	70.79
F8	0.961	0.046	0.958	0.001	0.859	0.846	0.959	0.001	1.029	0.958	0.039	50.08
F9	0.984	0.124	0.965	0.002	0.857	2.284	0.973	0.000	1.095	0.984	0.071	67.86
F10	0.969	0.152	0.964	0.002	0.884	2.822	0.969	0.000	0.919	0.969	0.246	81.99
F11	0.977	0.138	0.970	0.002	0.878	2.541	0.975	0.000	0.963	0.976	0.171	75.22
F12	0.985	0.077	0.985	0.001	0.887	1.421	0.986	0.000	0.961	0.984	0.097	53.00

AIC – Akaike Information Criterion.

F8 and F12, at 31.2, 21.73 and 37.61%, respectively. This can be attributed to the absence of the disintegrant action of HPMC, allowing for more sustained-release effects.

All the matrix tablets containing NTM (F1–F4) had values lower than 60%. There was no marked change in drug release as the polymer concentration increased from 5 to 10% (57.43 and 54.97% for F1 and F2, respectively). However, peak release was observed at a polymer concentration of 10% for matrix tablets F6 (65.36%) and F10 (74.64%). F10 represents the highest percentage drug release observed for all the matrix tablets. The percentage drug release was found to increase in the order MTM60 > MTM20 > NTM.

Table 7 shows the release parameters (correlation coefficient [r^2], diffusional release exponents [n] and kinetic constants) of matrix tablets F1–F12, obtained using zero order, first order, Higuchi, Hixson-Crowell and Korsmeyer release models. With the exception of the Higuchi model, all the models exhibited the greatest correlation values with the F12 matrix tablets. The highest correlation coefficient (0.986) was observed in the Hixson-Crowell model. The zero and first-order models both have r^2 values of 0.985, while the Korsmeyer-Peppas model showed 0.984. The highest r^2 value observed for the Higuchi model (0.910) occurred with F6 matrix tablets.

Matrix tablets F4, F5 and F6 had release exponent (n) values < 0.89 but > 0.45; thus indicating Non-Fickian transport. All the other matrix tablets exhibited n values > 0.89, indicating Super Case II transport. This indicates a combination of diffusion and erosion-controlled drug release.¹⁶

Table 6 shows the model-independent release parameters. The longest drug release times were observed with matrix tablets F4, F8 and F12, none of which contain HPMC, but which contain 10% (40 mg) of NTM, MTM20 and MTM60, respectively. There were no significant differences in $t_{25\%}$, $t_{50\%}$, $t_{75\%}$ and $t_{90\%}$ drug release between tablets F1–F3, F5–F7 or F9–F11.

Sustained-release action was strongest in the formulations containing MTM20, and this action was more pronounced in formulations F4, F8 and F12, which did not contain HPMC. The $t_{90\%}$ values were 29.78 h, 32.82 h and 19.50 h for F4, F8 and F12, respectively. Ranking for sustained-release action was in the order MTM20 > NTM > MTM60. There were no significant differences in MDT for any of the gum samples/matrix tablets.

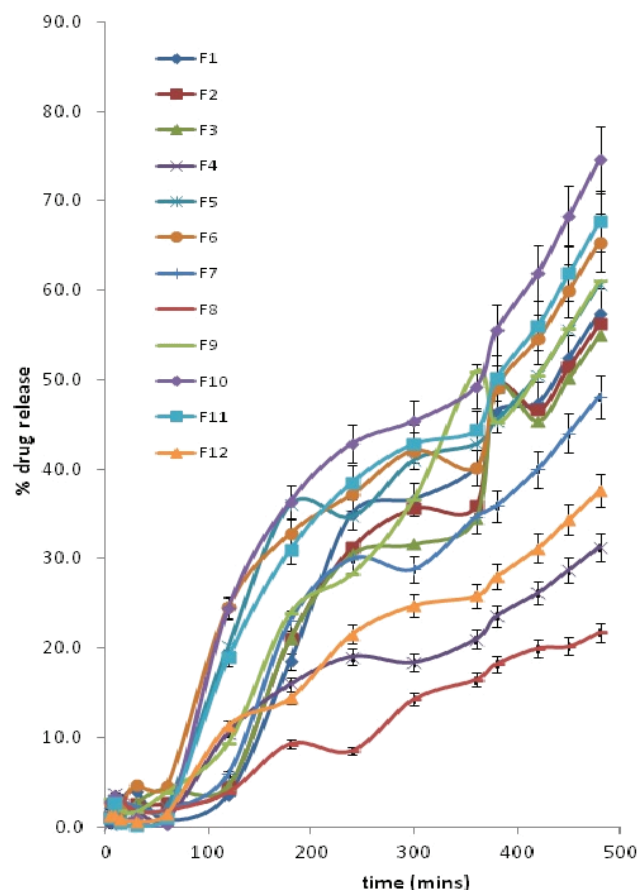


Fig. 2. Drug release profiles from naproxen matrix tablets

Conclusion

The flow properties of *Terminalia* gum were improved by microwave irradiation. Irradiation of native *Terminalia* gum for 20 seconds caused an improvement in its sustained-release activity. Bioadhesion improved with increasing polymer concentrations in formulations containing both *Terminalia* gum and HPMC. Irradiation of *Terminalia* gum generally improves its disintegrant action. A blend of the gum and HPMC could be directly compressed to give a simple, fast and consistent method of tablet production.

References

- Nimish S, Mewada RK, Tejal S. Application of biodegradable polymers in controlled drug delivery. Institute of Technology, Nirma University, Ahmedabad. 2011;382(481):8–10.
- Pandey R, Khuller GK. Polymer based drug delivery systems for mycobacterial infections. *Curr Drug Deliv*. 2004;1:195–201.
- Chamarthy SP, Pinal R. Plasticizer concentration and the performance of a diffusion-controlled polymeric drug delivery system. *Colloids Surf. A. Physiochem Eng Asp*. 2008;331:25–30.
- Roy H, Brahma CK, Nandi S, Parida KR. Formulation and design of sustained release matrix tablets of metformin hydrochloride: Influence of hypromellose and polyacrylate polymers. *Int J Applied Basic Med Res*. 2013;3:55–63. doi: 10.4103/2229–516X.112242.
- Sharma BR, Kumar V, Soni P. Ceric ammonium nitrate initiated graft copolymerization of acrylamide onto *Cassia tora* gum. *J Appl Polym Sci*. 2002;86:3250–3255.
- Odeniyi MA, Babalola AO, Ayorinde JO. Evaluation of *Cedrela* gum as a binder and bioadhesive component in ibuprofen tablet formulations. *Braz J Pharm Sci*. 2013;49(1):95–105.
- Parodi F. Microwave heating and the acceleration of polymerization processes. In: Wlochowicz A (ed) Polymers and liquid crystals. Proceedings of SPIE – The International Society for Optical Engineering 4017, p 2(1999).
- Bogdal D, Penczek P, Pielichowski J, Prociak A. Microwave assisted synthesis, crosslinking, and processing of polymeric materials. *Adv Polym Sci*. 2003, 163,193–263.
- Singh V, Tiwari A, Tripathi DN, Sanghi R. Microwave assisted synthesis of guar-g-polyacrylamide. *Carbohydr Polym*. 2004;58:1–6.
- Kaity S, Isaac J, Kumar PM, Bose A, Wong TW, Ghosh A. Microwave assisted synthesis of acrylamide graft ed locust bean gum and its application in drug delivery. *Carbohydr Polym*. 2013;98:1083–1094.
- Berressem P. The birth of new delivery systems. *Chem Br*. 1999;35:29–32.
- Adedokun MO, Ayorinde JO, Odeniyi MA. Compression, mechanical and release properties of a novel gum in paracetamol tablet formulations. *Curr Iss Pharm Med Sci*. 2014;27:187–194.
- Fell JT, Newton JM. Determination of tablet strength by diametrical compression test. *J Pharm Sci*. 1970; 59:688–691.
- Kafedjiiski K, Werle M, Föger F, Bernkop-Schnürch A. Synthesis and in vitro characterization of a novel poly(acrylic acid)-glutathione conjugate. *J Drug Del Sci Tech*. 2005;15:411–417.
- Emikpe BO, et al. *Ex-vivo* evaluation of the mucoadhesive properties of *Cedrela odorata* and *Khaya senegalensis* gums with possible applications for veterinary vaccine delivery. *SpringerPlus*. 2016;5:1289.
- Adetunji AO, Odeniyi MA, Itiola OA. Characterisation and controlled release properties of *Entandophragma angolense* gum in ibuprofen matrix tablets. *Farmacia*. 2015;63:57–64.
- Mokel J, Lippold B. Zero-order drug release from hydrocolloid matrices. *Pharm Res*. 1993;10:1066–1070.
- Odeniyi MA, Khan HN, Peh KK. Release and mucoadhesion properties of diclofenac matrix tablets from natural and synthetic polymer blends. *Acta Poloniae Pharmaceutica – Drug Res*. 2015;72:559–567.
- Costa P, Lobo JMS. Modeling and comparison of dissolution profiles. *Eur J Pharm Sci*. 2001;13:123–133.
- Bakre LG, Jaiyeoba KT. Studies on the physicochemical properties of *Abelmoschus esculentus* L. (Okra) pods – a potential tablet excipient. *Int J Biol Chem Sci*. 2007;3:448–456.
- Ayorinde JO, Odeniyi MA, Itiola OA. Evaluation of pharmaceutical and chemical equivalence of selected brands of diclofenac sodium tablets. *East Central African J Pharm Sci*. 2012;15:3–9.
- Alebiowu G, Itiola OA. Effects of pregelatinization of starch binders on the interacting variables acting on the mechanical properties of a paracetamol tablet formulation. *STP Pharm Sci*. 2002;12:379–383.
- British Pharmacopoeia (2010). Vol. 1. The Pharmaceutical Press, Her Majesty Stationery Office, London.
- Bamiro OA, Owoduni AS, Bakre LG, Uwaezuoke OJ. Evaluation of *Terminalia randii* Baker F. gum as a disintegrant in paracetamol tablet formulation. *J Chem Pharm Res*. 2014;6:155–159.
- Lawal MV, Odeniyi MA, Itiola OA. Effect of thermal and chemical modifications on the mechanical and release properties of paracetamol tablet formulations containing corn, cassava and sweet potato starches as filler-binders. *Asian Pacific J Trop Biomed*. 2015;5:576–580.
- Alebiowu G, Adeagbo AA. Disintegrant properties of a paracetamol tablet formulation lubricated with co-processed lubricants. *Farmacia*. 2009;57:500–510.

The role of chitin and chitosan in peripheral nerve reconstruction

Michał Bąk^{A–D}, Olga N. Gutkowska^{A,B,D}, Ewa Wagner^{B,C}, Jerzy Gosk^{A–C,E,F}

Department of Traumatology, Clinical Department of Traumatology and Hand Surgery, Wrocław Medical University, Wrocław, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;

D – writing the article; E – critical revision of the article; F – final approval of article

Polymers in Medicine, ISSN 0370-0747 (print), ISSN 2451-2699 (online)

Polim Med. 2017;47(1):43–47

Address for correspondence

Jerzy Gosk

E-mail: jerzy.gosk@umed.wroc.pl

Funding sources

none declared

Conflict of interest

none declared

Received on June 08, 2017

Revised on June 18, 2017

Accepted on July 03, 2017

Abstract

Chitin is a natural polysaccharide commonly found in nature and chitosan is its partially deacetylated derivative. The properties of both biopolymers allow their wide use in medicine and various industries. This paper presents the possibilities offered by chitin and chitosan for the creation of neurotubes utilized in peripheral nerve repair procedures. In the initial part of this manuscript, experimental studies on both polysaccharides carried out by numerous authors have been presented and their results have been discussed. Further, basic information on Reaxon® Nerve Guide, being the first chitosan tube approved for clinical use, is provided. Finally, existing limitations in the optimal use of chitosan tubes in peripheral nerve reconstruction have been pointed out. It is expected that modification of the properties of chitosan itself as well as enriching neurotubes with components of extracellular matrix, cells, growth factors and filaments will further improve the results of nerve regeneration obtained with chitosan-based nerve conduits.

Key words: artificial nerve conduit, chitosan nerve guide, nerve regeneration, nerve reconstruction

DOI

10.17219/pim/75653

Copyright

© 2017 by Wrocław Medical University

This is an article distributed under the terms of the
Creative Commons Attribution Non-Commercial License
(<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

Chitin is a biopolymer commonly found in the exoskeletons of arthropods, the shells of crustaceans and insect cuticles.^{1–3} It is a linear homopolymer composed of N-acetyl-D-glucosamine units that form β -(1 $\rightarrow$ 4)-linkages.^{2,3} Chitin is, after cellulose, the second most abundant polysaccharide in nature.⁴

Chitosan is obtained by the partial deacetylation of chitin. It is a polysaccharide composed of D-glucosamine and N-acetyl-D-glucosamine units linked through 1–4 glycosidic bonds.⁵ In nature, chitosan is found in some fungi.² It is commercially obtained by alkaline hydrolysis of chitin.³ The degree of acetylation of chitosan can range from 0 to 60%.³ Thanks to their advantageous properties, both chitin and chitosan are widely used in medicine and industry.^{1,6–8} These characteristics include: a “non-toxic character, biocompatibility, biodegradability, ease of creating various forms (films, sponges, fibres, hydrogels, porous scaffolds), chemical and enzymatic modifiability, antimicrobial properties, the capacity for controlled release of cytokines, extracellular matrix components and antibiotics, the ability to cause cell adherence, as well as to induce and maintain viability of tissue cells”.^{1,4–7} The above-listed features prompted research on the possible uses of chitin and chitosan in peripheral nerve reconstruction.

Experimental studies

Itoh et al. examined in experimental conditions the properties of chitosan tubes prepared from crab *Macrocheira kaempferi* tendons. The tubes had circular and triangular cross-sections. Some of the tubes having a triangular cross-section had also laminin and laminin peptides (CDPGYIGSR or CSRARKQAASIKVAVSAD) adsorbed, which enhanced migration and attachment of Schwann cells as well as neurite outgrowth.⁹ Rat sciatic nerves, in which 10 mm defects had been created, were used in the experiment. The defects were bridged with the above-described tubes, measuring 15 mm in length. Isografts were used in the control group. The investigation of the tubes 2–4 weeks after the implantation revealed tube infiltration with inflammatory cells and macrophages, which was associated with the initial period of the tube wall fragmentation process.⁹ After 6 weeks, the authors found evidence of nerve regeneration in both tubes made of unblended chitosan (circular and triangular) but it took place mainly in the center of the tube, whereas in the tubes enriched with laminin or laminin peptides, regeneration proceeded along the inner wall of the tube. However, in neither group was the degree of regeneration comparable to that obtained with the use of isografts.⁹ Another interesting conclusion drawn by the authors of the discussed study was that triangular tubes had higher mechanical strength than circular tubes. The latter had a higher tendency to collapse.⁹

The results obtained encouraged the Japanese researchers to continue their studies. Wang created a chitosan nonwoven nano/microfiber mesh tube with the use of an electro-spinning method.⁶ The bilayered tube contained an outer layer of chitosan film and an inner layer of chitosan nano/microfiber mesh. In this experiment on rat sciatic nerves with a 10 mm defect, the authors used:

1. Nano/microfiber mesh tubes with a deacetylation rate of 78 or 93%,
2. Bilayered tubes with a nano/microfiber mesh inner structure with a deacetylation rate of 78 or 93% and a film outer layer with deacetylation rate of 93%,
3. Film tubes with a deacetylation rate of 93%.

Isografts were used in the control group.⁶ At 5 weeks after the implantation, fragmentation of the nano/microfiber mesh began. This process was accompanied by an inflammatory response manifested by gathering of macrophages around the tube wall. A faster biodegradation rate was observed for the chitosan nano/microfiber mesh with a deacetylation rate of 78%. These tubes collapsed over time causing narrowing of the tube lumen. By contrast, chitosan nano/microfiber mesh tubes with a deacetylation rate of 93% kept their shape for as long as 15 weeks after the implantation. At the same time, the authors pointed out that the compressive strength of both the bilayered chitosan tube and the film tube was significantly greater than that of the chitosan nano/microfiber mesh.⁶ A conclusion can be drawn that the mechanical strength of the bilayered tube depended mainly on the film tube. On the other hand, when the functional results were analyzed, the highest number of myelinated axons and the greatest total axon area were found in the nano/microfiber mesh tubes with a deacetylation rate of 93%. Thanks to the electro-spinning method used in their preparation process, the mechanical properties of these tubes were sufficient to support regenerating axons. A single-layered wall made the exchange of nutrients and the excretion of metabolic waste products easier.⁶

The influence of the degree of acetylation of chitosan on its properties was also noted by other authors. Freier et al. studied the compressive strength of chitin gel tubes with an acetylation rate of 94% and chitosan tubes with an acetylation rate of 18, 3 and 1%.² The chitin tubes initially had high compressive strength but they soon lost it as their degradation proceeded. The compressive strength of the chitosan tubes rose with the decrease of acetylation rate.² According to the authors, a very low acetylation rate of chitosan is associated with high mechanical strength and a slower degradation process as well as increased viability and adhesion properties of lumbar dorsal root ganglion cells placed on the chitosan film.^{2,3} Freier et al. expressed the opinion that the main factor determining cell compatibility with chitosan is its charge, which depends on the amount and availability of amine groups.^{2,3} The charge density of chitosan increases with the decreasing degree of acetylation, leading to greater cell adhesion.^{2,3}

In the study on rat sciatic nerves with a 10 mm defect conducted by Haastert-Talini et al., it was determined that a degree of acetylation around 5% is optimal for nerve regeneration.¹⁰ Chitosan tubes with an acetylation rate of 2% were not able to sustain regenerating axons, whereas tubes with an acetylation rate of 20% succumbed to degradation too early and had low mechanical stability.¹⁰ Similarly, Gonzalez-Perez et al. in their study concluded that a degree of acetylation of chitosan of 5% favored nerve regeneration when compared to a lower degree of acetylation (2%).¹¹ The study was conducted on rat sciatic nerves with a 15 mm defect.¹¹

The beneficial effect on nerve regeneration can be enhanced not only by a modification of chitosan properties but also by enriching the neurotubes with certain substances, e.g. laminin, growth factors (glial cell line-derived nerve growth factor – GDNF) or cells (bone marrow stem cells – BMSCs).^{7,12,13} Laminin-1 is a natural extracellular matrix glycoprotein (molecular weight, 800 kDa), that enhances neural cell attachment, differentiation and neurite outgrowth.^{7,12} Certain domains (YIGSR, IKVAV, RGD) are responsible for these properties of laminin.^{7,9} Both laminin and the above-mentioned peptides were used in many experimental studies to enhance the chitin/chitosan effect on nerve regeneration.^{9,12,14} Continuing their research, the Japanese scientists investigated the possibility to further modify the properties of the tubes. They suggested introducing glycine spacers into the YIGSR sequence.⁷ After creation of the amino acid sequences CGGYIGSR and CG6YIGSR, they were covalently joined with a nano/microfiber mesh fabricated with the electro-spinning method. This structure created the inner layer of the tube. The outer layer was made of chitosan film. The experiment was conducted on rat sciatic nerves with a 15 mm defect with the use of the following tubes: 1) bilayered chitosan tube, 2) bilayered chitosan tube with CYIGSR sequence, 3) bilayered chitosan tube with CG2YIGSR sequence, 4) bilayered chitosan tube with CG6YIGSR sequence. Isografts were used in the control group. The autopsies were carried out after 5 and 10 weeks. In the first group (non-treated tube), the number of axons was limited and they could be found in the center of the tube. In the laminin-peptide-treated groups, the number of regenerating axons was higher and many of them adhered to the inner wall of the tube. The best results were obtained by increasing the spacer group length (CG6YIGSR), but still they were inferior to those in the control group (isograft).⁷

Patel et al. in their experimental studies, used chitosan tubes enriched not only with laminin, but also with glial cell line-derived nerve growth factor (GDNF).¹² GDNF provides trophic support to motor neurons, promotes axon regeneration and prevents the atrophy of motor neurons.^{15,16} Similarly to laminin, GDNF has the capacity to relieve neuropathic pain.^{17,18}

Three types of chitosan tubes were used in this study: 1) C nerve guide (unblended chitosan guide); 2) LC nerve guide (laminin-chitosan guide); 3) GLC nerve guide (GDNF-laminin-chitosan guide). Autografts were used in the control group. The experiment was carried out on rat sciatic nerves with a 10 mm defect. After 12 weeks of observation, it was determined that functional recovery results were comparable in the LC and GLC groups and much better than in the C group, in which unblended chitosan nerve guide was used. These findings confirmed previous conclusions made by the same authors regarding the limited potential for nerve regeneration with the use of chitosan tubes alone.¹⁹

A different modification of chitosan-based tube was proposed by Hsu et al., who created a laminin-coated chitosan multi-walled nerve conduit combined with bone marrow stem cells (BMSCs).¹³ In this experiment, 10 mm defects in rat sciatic nerves were bridged with: 1) empty silicone conduit, 2) laminin (LN)-modified chitosan scaffold in silicone conduit, 3) laminin (LN)-modified chitosan scaffold with BMSCs in silicone conduit. The LN-modified chitosan film (10 × 10 mm) was rolled up and put into the silicone conduit measuring 1.57 (inner diameter) × 2.41 (outer diameter) × 14 mm. Sciatic nerve stumps were inserted into the conduit to a depth of 2 mm. Autopsies were carried out after 16 weeks. In group 2, the tube was surrounded by granulation hyperplasia tissue, which was mainly composed of eosinophils and macrophages.¹³ No such phenomenon was observed in group 3, in which the tubes were enriched with BMSCs. The authors concluded: “BMSCs suppressed neuronal cell death and promoted regeneration by suppressing the inflammatory and fibrotic response induced by chitosan after long-term implantation”.¹³

Lauto et al. suggested another use of chitosan in peripheral nerve repair.²⁰ The authors developed a novel adhesive composed of chitosan, indocyanine green, acetic acid and water. This chitosan adhesive was then used in an experimental study on rats to repair tibial nerve defects with the use of a laser. The laser fluence applied to the operated nerves did not exceed 46 J/cm². In the course of the experiment, good quality nerve repair was achieved, with tensile strength of 12.5±2.6 kPa. Thus, the connection between the nerve stumps was stronger than provided by fibrin glue, the adhesive strength of which is estimated at ~8 kPa.²⁰

The authors highlighted a number of advantages of chitosan that make it especially effective in this type of nerve repair procedures, including its insolubility in physiological fluids as opposed to fibrin glue, its adhesiveness prior to laser activation and its hydrophilic properties.²⁰ The chitosan adhesive is based on polysaccharide, which eliminates the risk of viral infection, unlike in the case of hemo-derived proteins.²⁰

Clinical uses of chitosan

A chitosan-based nerve conduit under the name Reaxon[®] Nerve Guide manufactured by Medovent GmbH (Mainz, Germany), in accordance with the international standard DIN EN ISO 13485, was launched onto the market in June, 2014. The length of the tube is 30 mm, with a range of inner diameter sizes available: 2.1, 3.0, 4.0, 5.0 and 6.0 mm. These diameters are observed in the dry state of the tube and increase by 0.1 to 0.4 mm after hydration. Flexibility and resistance to collapse are amongst the advantages of the tube, as declared by the manufacturer. A transparent tube wall makes insertion of nerve stumps easier and its hydrogel character facilitates suturing nerve stumps to the tube. Electrostatic interaction between the positively-charged surface of Reaxon[®] Nerve Guide and negatively-charged biomolecules and cell components favors nerve regeneration. Reaxon[®] Nerve Guide is designed to aid in the repair of nerve defects not exceeding 26 mm in clinical settings. Intraoperatively, after having chosen an adequate inner tube diameter, the tube is placed in sterile saline solution for at least 10 min. The nerve stumps are connected to the tube with the use of monofilament non-absorbable size 8–0 sutures. The tube is filled with saline solution.²¹

Medovent GmbH has run 2 clinical investigation programs dedicated to the clinical uses of Reaxon[®] Nerve Guide since 2015. The first program, entitled “A controlled, randomized, comparison, blind evaluation of repair of digital nerve lesions in men using an implanted Reaxon[®] Nerve Guide”, will comprise 76 patients with traumatic digital nerve defects, not exceeding 26 mm in length. The estimated study completion date is May 2018.

The other study, designed as a randomized, double-blind, controlled, multicenter trial, was originated in February, 2015. The study is being conducted concurrently at 3 trauma centers in Ludwigshafen, Frankfurt am Main and Bochum and comprises cases of acute digital nerve injuries (up to 72 h from the injury). One hundred patients are planned to be included in the study. In a half of them, a simple “end-to-end” repair will be conducted, while in the other half a 1 cm-long chitosan tube with a diameter of 2.1 mm will additionally be used. Sutures 9–0 will be used in both groups. The outcomes will be evaluated after 3, 6, 12 and 24 months. The duration of the project is estimated at 36 months, until the completion of all follow-up examinations.²²

Reaxon[®] Nerve Guide was also used by Fornasari et al. in experimental studies on rats. The authors examined the influence of the presence of skeletal muscle fibers on nerve regeneration.²³ The rats were divided into 2 groups. In one group, an 8 mm median nerve defect was repaired with a 10 mm-long chitosan tube. In the other group, a longitudinal piece of the pectoralis major muscle was inserted in each tube. Autografts were used in the control group. Both early (7th, 14th and 28th day after the op-

eration) and late (3rd month after the operation) control examinations were performed. The authors presumed that the presence of muscle tissue inside the tube, being a source of neuregulin 1 (a factor enhancing Schwann cell livability and activity) would have a pronounced positive effect on the regeneration process. It turned out that with short defects, both a chitosan tube alone and the tube enriched with skeletal muscle tissue were effective in promoting the return of nerve function.²³

Summary

It is beyond doubt that chitin, and even more so chitosan, constitute an excellent basis for creation of an optimal neurotube. The possibility to change the degree of acetylation of chitosan makes possible the modification of its mechanical properties and the degradation rate of the tube.⁶ Modern technological capabilities enable the creation of a tube made of chitosan fibers measured on the nanometric scale.⁶ As a result, a 3-dimensional porous microstructure can be obtained that imitates extracellular matrix (ECM). The porosity of the tube wall makes the exchange of nutrients and the excretion of metabolic waste products easier.^{6,7} Moreover, such a nano/microfibrous structure has a high surface area-to-volume ratio. Such a large surface area allows effective binding with other substances, e.g. laminin – a protein that is a natural constituent of extracellular matrix, cells (BMSCs) and growth factors (GDNF).^{7,12,13}

There are several reasons for enriching chitosan tubes with other substances. Firstly, the properties of the added substances accelerate the regeneration processes. Secondly, they minimize the side effects of chitosan degradation. Chitosan as a polymer and its short chains, unlike its degradation fragments, do not cause an inflammatory response.^{24,25} Hsu et al. demonstrated that the chitosan fragments produced by degradation were able to induce inflammation, which caused apoptosis of newly generated cells, and proliferation of fibrous tissue around the conduit.¹³ Experimental studies have resulted in launching a chitosan tube onto the market for clinical use: Reaxon[®] Nerve Guide.^{21,26} It allows the repair of short nerve defects, when the nerve diameter does not exceed 6.0 mm. The repair of nerve defects in nerves with a larger diameter still constitutes a challenge.^{27,28} Obtaining satisfactory regeneration in such cases will only be made possible with the use of a technologically advanced neurotube. An “ideal nerve conduit” is expected to have the following characteristics: biodegradability, porosity and permeability of the tube wall, presence of an inner scaffold made of fibers or filaments, the capacity to sustain cell livability and promote cell migration, the ability to secrete growth factors and electrical conductivity.^{29–32} As the analysis of the presented studies indicates, chitosan, thanks to its versatile properties, could be a component of such an ideal neurotube.

References

- Muzzarelli RAA. Chitins and chitosans for the repair of wounded skin, nerve, cartilage and bone. *Carbohydr Polym.* 2009;76:167–182.
- Freier T, Montenegro R, Shan Koh H, Shoichet MS. Chitin-based tubes for tissue engineering in the nervous system. *Biomaterials.* 2005;26:4624–4632.
- Freier T, Koh HS, Kazazian K, Shoichet MS. Controlling cell adhesion and degradation of chitosan films by N-acetylation. *Biomaterials.* 2005;26:5872–5878.
- Crompton KE, Goud JD, Bellamkonda RV, et al. Polylysine-function-alised thermoresponsive chitosan hydrogel for neural tissue engineering. *Biomaterials.* 2007; 28:441–449.
- Lu G, Kong L, Sheng B, Wang G, Gong Y, Zhang X. Degradation of covalently cross-linked carboxymethyl chitosan and its potential application for peripheral nerve regeneration. *Eur Polym J.* 2007;43:3807–3818.
- Wang W, Itoh S, Matsuda A, et al. Influences of mechanical properties and permeability on chitosan nano/microfiber mesh tubes as a scaffold for nerve regeneration. *J Biomed Mater Res A.* 2008, 84, 557–566.
- Wang W, Itoh S, Matsuda A, et al. Enhanced nerve regeneration through a bilayered chitosan tube: the effect of introduction of glycine spacer into the CYIGSR sequence. *J Biomed Mater Res A.* 2008;85:919–928.
- Mazurek P, Kuliński S, Gosk J. Możliwości wykorzystania chityny i chitozanu w leczeniu ran. *Polim Med.* 2013;43:297–302.
- Itoh S, Suzuki M, Yamaguchi I, et al. Development of a nerve scaffold using a tendon chitosan tube. *Artif Organs.* 2003;27:1079–1088.
- Haastert-Talini K, Geuna S, Dahlin LB, et al. Chitosan tubes of varying degrees of acetylation for bridging peripheral nerve defects. *Biomaterials.* 2013;34:9886–9904. doi: 10.1016/j.biomaterials.2013.08.074.
- Gonzalez-Perez F, Cobiañchi S, Geuna S, et al. Tubulization with chitosan guides for the repair of long gap peripheral nerve injury in the rat. *Microsurgery.* 2015;35:300–308. doi: 10.1002/micr.22362.
- Patel M, Mao L, Wu B, Vandevord PJ. GDNF-chitosan blended nerve guides: a functional study. *J Tissue Eng Regen Med.* 2007;1:360–367.
- Hsu SH, Kuo WC, Chen YT, et al. New nerve regeneration strategy combining laminin-coated chitosan conduits and stem cell therapy. *Acta Biomater.* 2013;9:6606–6615. doi: 10.1016/j.actbio.2013.01.025.
- Suzuki M, Itoh S, Yamaguchi I, et al. Tendon chitosan tubes covalently coupled with synthesized laminin peptides facilitate nerve regeneration in vivo. *J Neurosci Res.* 2003;72:646–659.
- Jubran M, Widenfalk J. Repair of peripheral nerve transections with fibrin sealant containing neurotrophic factors. *Exp Neurol.* 2003;181:204–212.
- Chen ZY, Chai YF, Cao L, Lu CL, He C. Glial cell line-derived neurotrophic factor enhances axonal regeneration following sciatic nerve transection in adult rats. *Brain Res.* 2001;902:272–276.
- Grimpe B, Silver J. The extracellular matrix in axon regeneration. *Prog Brain Res.* 2002;137:333–349.
- Wang R, Guo W, Ossipov MH, Vanderah TW, Porreca F, Lai J. Glial cell line-derived neurotrophic factor normalizes neurochemical changes in injured dorsal root ganglion neurons and prevents the expression of experimental neuropathic pain. *Neuroscience.* 2003;121:815–824.
- Patel M, Vandevord PJ, Matthew H, Wu B, DeSilva S, Wooley PH. Video-gait analysis of functional recovery of nerve repaired with chitosan nerve guides. *Tissue Eng.* 2006;12:3189–3199.
- Lauto A, Foster LJ, Avolio A, et al. Sutureless nerve repair with laser-activated chitosan adhesive: A pilot in vivo study. *Photomed Laser Surg.* 2008;26:227–234. doi: 10.1089/pho.2007.2131.
- Medovent. Reaxon® Nerve Guide-Medovent. http://medovent.de/wp-content/uploads/2015/12/REAXON_GB_mail.pdf. Accessed August 28, 2017.
- Neubrech F, Heider S, Harhaus L, Bickert B, Kneser U, Kremer T. Chitosan nerve tube for primary repair of traumatic sensory nerve lesions of the hand without a gap: study protocol for a randomized controlled trial. *Trials.* 2016;17:48. doi: 10.1186/s13063-015-1148-5.
- Fornasari BE, Gambarotta G, Ronchi G, et al. Chitosan tubes enriched by skeletal muscle for peripheral nerve regeneration. 6th Vienna Symposium on Surgery of Peripheral Nerves. 17–19.03.2017. Poster; 16.
- Reese TA, Liang HE, Tager AM, et al. Chitin induces accumulation in tissue of innate immune cells associated with allergy. *Nature.* 2007;447:92–96.
- Baldrick P. The safety of chitosan as a pharmaceutical excipient. *Regul Toxicol Pharmacol.* 2010;56:290–299. doi: 10.1016/j.yrtph.2009.09.015.
- Shapira Y, Tolmasov M, Nissan M, et al. Comparison of results between chitosan hollow tube and autologous nerve graft in reconstruction of peripheral nerve defect: An experimental study. *Microsurgery.* 2016;36:664–671. doi: 10.1002/micr.22418.
- Gosk J, Mazurek P, Reichert P, Wnukiewicz W, Rutowski R. Możliwości wykorzystania materiałów nie ulegających degradacji jako tub w rekonstrukcjach nerwów obwodowych. *Polim Med.* 2010;40:3–8.
- Gosk J, Urban M, Ratajczak K, Wiacek R, Rutowski R. Zastosowanie syntetycznych polimerów ulegających biodegradacji w rekonstrukcjach nerwów obwodowych. *Polim Med.* 2010;40:3–9.
- Jiang X, Lim SH, Mao HQ, Chew SY. Current applications and future perspectives of artificial nerve conduits. *Exp Neurol.* 2010;223:86–101. doi: 10.1016/j.expneurol.2009.09.009.
- de Ruiter GC, Malessy MJ, Yaszemski MJ, Windebank AJ, Spinner RJ. Designing ideal conduits for peripheral nerve repair. *Neurosurg Focus.* 2009;26:E5. doi: 10.3171/FOC.2009.26.2.E5.
- Ichihara S, Inada Y, Nakamura T. Artificial nerve tubes and their application for repair of peripheral nerve injury: An update of current concepts. *Injury.* 2008;39 Suppl:S29–S39. doi: 10.1016/j.injury.2008.08.029.
- Johnson EO, Soucacos PN. Nerve repair: Experimental and clinical evaluation of biodegradable artificial nerve guides. *Injury.* 2008;39 Suppl:S30–S36. doi: 10.1016/j.injury.2008.05.018.

Chitosan and its composites: Properties for use in bone substitution

Marek Stępniewski^{A–D}, Jacek Martynkiewicz^{A–D}, Jerzy Gosk^{A–C,E,F}

Clinical Department of Traumatology and Hand Surgery, Wrocław Medical University, Wrocław, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;

D – writing the article; E – critical revision of the article; F – final approval of article

Polymers in Medicine, ISSN 0370-0747 (print), ISSN 2451-2699 (online)

Polim Med. 2017;47(1):49–53

Address for correspondence

Marek Stępniewski

E-mail: marek.stepniewski@wp.pl

Funding sources

none declared

Conflict of interest

none declared

Received on July 18, 2017

Revised on August 06, 2017

Accepted on August 20, 2017

Abstract

For many years, research has been carried out on finding an ideal bone substitute. Chitosan (CTS) is a naturally occurring polysaccharide, obtained mainly from, inter alia, the shells of crustaceans. It is characterized by its high level of biocompatibility, biodegradability and antimicrobial properties as well as its support in the healing of wounds. Chitosan, due to its ability to form porous structures, can be used in the production of scaffolds used in the treatment of bone defects. There are numerous studies on the use of CTS in combination with other substances which aim to improve its biological and mechanical properties.

The combination of chitosan with the calcium phosphate hydroxyapatite (HAp) has been extensively tested. The objective of the current studies is to verify the properties of scaffolds consisting of chitosan and other substances like polybutylene succinate, human bone marrow mesenchymal stem cells (hBMSCs), collagen, alginate, transforming growth factor – β (TGF- β), insulin-like growth factor (IGF), platelet-derived growth factor (PDGF) or bone morphogenetic proteins (BMP). The aim of the current research is to develop a scaffold with sufficiently good mechanical properties. Trials are underway with many of the biological and synthetic components affecting the biological properties of chitosan. This will allow for the creation of a substitute that fully meets the conditions for an ideal artificial bone.

Key words: chitosan, chitin, scaffold, bone substitution

DOI

10.17219/pim/76517

Copyright

© 2017 by Wrocław Medical University

This is an article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

The ideal material used to supplement bone defects should possess 4 properties: osteoconduction, osteoinduction, osteointegration and osteogenesis. Osteoconduction is the ability of a material to promote bone growth by creating appropriate physical conditions, such as the porous spatial structure through which osteogenic or neoplastic cells penetrate the bone. Osteoinduction involves the exertion of influence on stem cells, targeting them to differentiate into osteoblasts, which are bone-forming cells. The most active group of osteoinductive factors are bone morphogenetic proteins (BMP). Osteointegration is the direct bonding of a new living bone with the bone substitute and as well as the gradual replacement of the substitute with living bone. Osteogenesis is the formation of new bone by osteogenic cells.¹

Osteointegration is a particularly important attribute of a graft, which significantly influences the final effect. Usually, this process is slow and does not always complete the graft remodeling process. Many factors affect the effectiveness of this joining in which the graft-forming material plays a crucial role, possessing properties of porosity and affecting the quality of the bone at the location of the graft's implantation.

There are several phases in the grafting process. In the initial phase, the transplant is filled with flowing blood on which basis the hematoma develops. Inflammatory cells and growth factors are introduced through this blood flow. Then there is a dilatation phase, in which, by the formation of new blood vessels and connective tissue, the graft initially attaches to the bone tissue. In subsequent stages the osteoclasts resorb elements of the graft where, in place of the osteocytes, new bone is formed, connecting both elements permanently. The last phase is the remodeling of the newly formed bone, leading to the rebuilding of the natural character of bones.

In orthopedics, 3 types of transplants are used for the repair of bone defects: autographs, allografts and bone substitutes. The reconstruction process is most effective in an autograft, i.e. bone graft. Its structure is porous, allowing easy penetration of new bone cells as well as new vessels. Autograft cells are mostly covered by osteoblasts that exhibit traits of osteoconductivity and osteoinductance. The most common graft location is the iliac crest. This is a perfect graft, but due to the limited amount of tissue available, the pain involved, the possibility of infection and increased blood loss or prolonged surgery time, other forms of bone substitution are also used.²

The most common alternative to self-bone grafting is allografts, i.e. grafts of material from a deceased donor. They can be prepared in any shape or size. However, besides providing the spatial structure of grafts, they do not meet the requirements of osteoinductivity, which may result in a weakened osteointegration.³

Due to the limitations of natural bone grafts, synthetic bone substitutes have been introduced.

Bone substitutes can be divided into 3 generations, depending on the degree of integration by the recipient's bone. The first generation includes pure metals (stainless steel, titanium), metal alloys (aluminum, zirconium) and polymers (silicone, polypropylene, polymethyl methacrylate). This group of grafts often develops a fibrous layer on the bone contact surface, which may lead to a lack of full osteointegration and a secondary loosening of the graft. The second generation of substitutes is coated with an additional supporting layer to prevent the formation and deposition of connective tissue on the graft and thereby facilitating complete osteointegration. This group includes hydroxyapatite, calcium metaphosphate and bioactive glass. The third generation uses a material most closely related to the natural structure of bone and is characterized by high osteoconductivity and bioactivity (osteoinductive and biodegradable).⁴

Properties of bone substitutes

One of the important properties of bone substitutes is biocompatibility. Fully biocompatible materials, i.e. materials that do not cause any bodily inflammatory reaction, either local or general, are used during the synthesis of these bone substitutes.

The porosity of the preparation is also very important. The greater the porosity, the easier it is for the new cells to penetrate. At the same time, the nature of the structure influences its mechanical strength: namely the greater the porosity, the lower the strength. The individual mechanical needs should be taken into account while preparing bone substitutes. One of the solutions is the use of "micro & nano" technology, which allows for the graft to have a structure with different pore diameters, simultaneously providing good osteointegration and mechanical strength.^{5,6}

The biodegradability of the bone substitute material must go hand in hand with maintaining the mechanical properties of the substitute, which means that it cannot be too rapid. Degradation of the graft before complete bone remodeling can lead to a weakened structure and consequent fractures within the graft. The risk of fractures can be reduced by strengthening the osteoinduction. To achieve this, the substitute is combined with a progenitor and osteoblast stimulating agents, which lead to an increased production of bone matrix. Among other things, the following are used: transforming growth factor – β (TGF- β), insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), and bone morphogenetic proteins (BMP).⁶

Chitosan

Chitin is one of the most common polysaccharides found in nature. It occurs naturally in arthropods, sponges and corals, as well as in other organisms. Its discoverer,

H. Brancota, was responsible for isolating chitin for the first time in 1811. The deacetylated form of chitin, called chitosan, was described by Rouget in 1859.

For industrial purposes, chitin is mainly obtained from marine invertebrates such as crabs, shrimp or krill by a process of chemical hydrolysis. The first stage is demineralization, i.e. shredding of crustacean armor as well as CaCO_3 deprivation (under the influence of HCl). The next step is deproteinization with a NaOH solution and discoloring with a KMnO_4 solution. This is how chitosan is obtained from the chitin that has undergone the deacetylation process. Alternatively, there is an enzymatic hydrolysis process using, inter alia, lysozymes or neutral proteases.⁷ The degree of deacetylation and molecular weight of chitosan depend on the substrate used as well as the method of production. The enzymatic method allows CTS to have higher molecular weights than other traditional methods. From the *Scopulariopsis brevicaulis* mushroom, the average CTS weight using the enzymatic method is 267.97 kDa while the traditional chemical method yields 84.04 kDa.⁷

Properties of chitosan

Because of its biological properties and the constantly renewable natural resource chitosan, in the form of chitin, has been a subject of scientific research for over 30 years in the field of medicine.

Chitosan exhibits several important bio-material properties. It is biocompatible, biodegradable, hydrophilic and non-toxic. It has hemostatic properties. Its porous structure facilitates penetration and binding to other cells, especially bone cells. It affects cells involved in the bone-forming process by activating macrophages and stimulating fibroblasts, and it also captures and binds to growth agents as well as supports the processes of angiogenesis.^{8,9} Chitosan has a cationic nature,¹⁰ which allows it to bind to negatively charged molecules, such as erythrocytes or thrombocytes, activating an extrinsic coagulation pathway.¹¹ Chitosan also exhibits antimicrobial properties. The cationic nature makes it possible to connect with the walls of *Staphylococcus aureus* species. This leads to damage as well as to the inhibition of the mRNA bacteria's synthesis. It may also be a carrier for various therapeutic substances. It is used as a carrier of silver ions in the production of modern wound dressings.^{12,13}

The ability to synthesize chitosan in various forms, i.e. paste membranes, sponges, fibers and spatial porous structures, such as a scaffold, is especially utilized in orthopedics.^{14,15} When supplementing bone defects, the most significant effect is through the use of spatial graphs obtained from chitosan.^{16,17}

Composite chitosan-hydroxyapatite

Insulated chitosan does not meet all the requirements of the ideal graft, but when mixed with other composites, it comes closer to having the properties of bone.

The combination of chitosan with the calcium phosphate hydroxyapatite (HAp) has been extensively tested. HAp is one of the most stable forms of calcium phosphate. It occurs naturally in the bones, making up 60–65% of inorganic bone components. In orthopedics, HAp has been applied as a coating for metal implants (e.g. hip endoprosthesis). The bone in contact with the HAp-coated implant begins to grow and gradually penetrates the pores, improving the mechanical attachment of the implant. HAp is also used in the supplementation of bone defects. Due to its poor mechanical properties, the grafts are limited to small sizes.¹⁸

CTS/HAp composites are completely non-toxic and exhibit a significant increase in osteoblast activity and their deposition/penetration of the composite.^{19–22} There are many methods for the synthesis of CTS/HAp, ranging from the simple mixing of natural HAp with CTS by means of freezing, lyophilization and hybridization as well as the use of state-of-the-art methods such as electrospinning. The method used affects the quality, especially the mechanical strength of the resulting scaffold, which depends on the bond of $-\text{NH}_2$ and $-\text{OH}$ groups of chitosan with Ca^{2+} hydroxyapatite and the ratio of CTS to HAp.^{20,23}

CTS/HAp composites are characterized by significantly better mechanical properties than both products individually. The highest compression strength of the CTS/HAp scaffold, 119.86 MPa, was obtained by the use of 30:70 CTS:HAp.²⁴ Joining of the CTS reduces the compression strength of the graft and the higher the molecular weight of the chitosan the higher the compressive strength.²⁵

Increasing the HAp content increases the compression strength. This is related to a lower number of bonds between CTS and HAp.²⁶ The temperature at which the synthesis of the molecules occurs, the higher the temperature, the stronger the binding, is also significant. The aquatic environment affects the decrease in mechanical properties. HAp/CTS/composite carboxymethylcellulose (40%/30%/30%), is characterized by a compression strength of 40 MPa under dry conditions and 12 MPa in an aqueous medium.²⁷

CTS/HAp scaffolds are characterized by their pore structure, averaging 100–200 μm , while allowing free deposition and migration of osteoblasts (averaging 10–30 μm) deep into the composite. It has been observed that osteoblasts embedded in the CTS/HAp scaffolds are activated after 30 min and after 5 days they will start to aggregate the bone reconstruction process.²⁸

Kawakami et al. studied the in vivo effect of CTS/HAP self-hardening paste through its application on the surface after removal of the periosteum. New bone formation was observed after 1 week and continued during a 20-week follow-up. This study confirmed the osteoconductivity of chitosan.³⁵

To assess the biological activity of the scaffolds, a level of alkaline phosphatase activity (FA) is used, which increases with increased osteoblast differentiation as well as during the early stage of bone formation. CTS/HAP composite produces a significantly higher FA growth activity than chitosan on its own. The highest FA activity was observed in composites containing 30–40% HAP. The use of grafts with a lower HAP content was linked with a lower FA activity.^{28,29}

Other composites containing chitosan

In order to improve the biological properties of chitosan, other associations have also been used, e.g. polybutylene succinate and bone marrow stem cells. Human bone marrow mesenchymal stem cells (hBMSCs) accelerate the rate of bone formation. Costa Pinto et al. investigated the effect of chitosan-based scaffold cultured with hBMSCs on the surrounding bone. Eight weeks after implantation in the location of the cranial defect in the skull coatings of nude mice, grafts were collected for mikroCT testing. Very good integration with the surrounding tissue as well as significant bone formation was observed.³⁰

Chitosan composites containing collagen and β -glycerophosphate are 3 times more rigid than pure chitosan. The osteogenic properties of such scaffolds are also better.³¹ Adding of alginate increases the activity of osteoblasts.³² CTS/HAP composite mixed with RGD peptide (ARG-GLY-ASP) has a stronger osteoconductivity.³³

Research is also being conducted on the possibility of using various scaffold growth agents. Osteogenic activity was greatly increased by insulin-like growth agent-1 (IGF-1), with slightly less bone morphogenetic protein-2 (BMP-2). Nande et al. investigated and compared the effectiveness of porous chitosan, alone and in combination with IGF-1 and BMP-2 in the healing of rabbit tibias with bone defects. Radiologically, evidence of radiodensity in the bone defect area was observed after the 60th day (started on the 30th day) in the rabbit group with IGF-1 and BMP-2 and in the 90th day in the chitosan-only group. Histological observation depicted better osteoblastic proliferation, vascularization and reticular network in the group with IGF-1.³⁴

Also, the addition of platelet-rich plasma (PRP) to the final scaffold was beneficial for osteogenesis. Bi et al. injected tricalcium phosphate/chitosan, in combination with autologous platelet-rich plasma, into the tibial bone defect of a goat. After 16 weeks, complete bone regeneration was observed.³²

Conclusions

The results of many studies carried out on bone substitutes show promising results on the safety and efficacy of chitosan-based scaffolds. Compound composites of chitosan and biocompatible polymers or bioresorbable ceramics may in the future fulfil the requirements of the ideal artificial bone graft. CTS/HAP composites are characterized by good osteoconduction, osteoinductivity and stimulation of osteogenesis. The aim of current research is to develop a scaffold with sufficiently good mechanical properties. Trials are underway with many of the biological and synthetic components affecting the biological properties of chitosan. This will allow for the creation of a substitute that fully meets the conditions for an ideal artificial bone.

References

- Greenwald AS, et al. Bone-graft substitutes: facts, fictions, and applications. *J Bone Joint Surg Am.* 2001;83-A Suppl 2 Pt 2:98-103.
- Stevens B, Yang Y, Mohandas A, Stucker B, Nguyen KT. A review of materials, fabrication methods, and strategies used to enhance bone regeneration in engineered bone tissues. *J Biomed Mater Res B.* 2008;85(2):573–582.
- Moore WR, Graves SE, Bain GI. Synthetic bone graft substitutes. *ANZ J Surg.* 2001;71(6): 354–361.
- Amini AR, Laurencin CT, Nukavarapu SP. Bone tissue engineering: recent advances and challenges. *Crit Rev Biomed Eng.* 2012;40(5):363–408.
- Polo-Corralles L, Latorre-Esteves M, Ramirez-Vick JE. Scaffold Design for Bone Regeneration. *J Nanosci Nanotechnol.* 2014;14(1):15–56.
- Rivera-Chacon DM, et al. Fibronectin and vitronectin promote human fetal osteoblast cell attachment and proliferation on nanoporous titanium surfaces. *J Biomed Nanotechnol.* 2013;9(6):1092–1097.
- Cai J, et al. Enzymatic preparation of chitosan from the waste *Aspergillus niger* mycelium of citric acid production plant. *Carbohydr Polymers.* 2006;64:151–157.
- Muzzarelli RAA. Chitins and chitosan for the repair of wound skin, nerve, cartilage and bone. *Carbohydr Polym.* 2009;76(2):167–182.
- Dai T, Tanaka M, Huang YY, Hamblin MR. Chitosan preparations for wounds and burns: antimicrobial and wound healing effects. *Expert Rev Anti Infect Ther.* 2011;9(7):857–879.
- Yang TL. Chitin – based materials in tissue engineering: Applications in soft tissue and epithelial organ. *Int J Mol Sci.* 2011;12(3):1936–1963.
- Cheung RCF, Ng TB, Wong JH, Chan WY. Chitosan: An update on potential biomedical and pharmaceutical applications. *Mar Drugs.* 2015;13(8):5156–5186.
- Guo R, Xu S, Ma L, Huang A, Gao C. Enhanced angiogenesis of gene-activated dermal equivalent for treatment of full thickness incisional wounds in a porcine model. *Biomaterials.* 2010;31(28):7308–7320.
- Liu X, Ma L, Liang J, Zhang B, Teng J, Gao C. RNAi functionalized collagen-chitosan/silicone membrane bilayer dermal equivalent for full thickness skin regeneration with inhibited scarring. *Biomaterials.* 2013;34(8):2038–2048.
- Muzzarelli RA, et al. Osteoconductive properties of methylpyrrolidone chitosan in an animal model. *Biomaterials.* 1993;14(12):925–929.
- Muzzarelli RA, et al. Stimulatory effect on bone formation exerted by a modified chitosan. *Biomaterials.* 1994;15(13):1075–1081.
- Costa-Pinto AR, et al. Osteogenic differentiation of human bone marrow mesenchymal stem cells seeded on melt based chitosan scaffolds for bone tissue engineering applications. *Biomacromolecules.* 2009;10(8):2067–2073.
- Tuzlakoglu K, Alves CM, Mano JF, Reis RL. Production and characterization of chitosan fibers and 3-D fiber mesh scaffolds for tissue engineering applications. *Macromol Biosci.* 2004;4(8):811–819.
- Kim SK, Mendis E. Bioactive compounds from marine processing byproducts – A review. *Food Res Int.* 2006;39(4):383–393.

19. Di Martino A, Sittering M, Risbud MV. Chitosan: A versatile bio-polymer for orthopaedic tissue-engineering. *Biomaterials*. 2005;26(30):5983–5990.
20. Teng SH, Lee EJ, Yoon BH, Shin DS, Kim HE, Oh JS. Chitosan/nanohydroxyapatite composite membranes via dynamic filtration for guided bone regeneration. *J Biomed Mater Res A*. 2009;88(3):569–580.
21. Thein-Han W, Misra R. Biomimetic chitosan – nanohydroxyapatite composite scaffolds for bone tissue engineering. *Acta Biomater*. 2009;5(4):1182–1197.
22. Xianmiao C, Yubao L, Yi Z, Li Z, Jidong L, Huanan W. Properties and in vitro biological evaluation of nano-hydroxyapatite/chitosan membranes for bone guided regeneration. *Mater Sci Eng C*. 2009;29(1):29–35.
23. Yamaguchi I, et al. Preparation and microstructure analysis of chitosan/hydroxyapatite nanocomposites. *J Biomed Mater Res*. 2001;55(1):20–27.
24. Li Z, Yubao L, Aiping Y, Xuelin P, Xuejiang W, Xiang Z. Preparation and in vitro investigation of chitosan/nano-hydroxyapatite composite used as bone substitute materials. *J Mater Sci Mater Med*. 2005;16(3):213–219.
25. Li Z, Yubao L, Aiping Y, Xuelin P, Xuejiang W, Xiang Z. Bioabsorbable chitosan/hydroxyapatite composite rod for internal fixation of bone fracture prepared by in situ precipitation. *Acta Polym. Sin*. 2002;6:828–833.
26. Liuyun J, Yubao L, Chengdong X. A novel composite membrane of chitosan-carboxymethyl cellulose polyelectrolyte complex membrane filled with nano-hydroxyapatite I. Preparation and properties. *J Mater Sci Mater Med*. 2009;20(8):1645–1652.
27. Aronow MA, Gerstenfeld LC, Owen TA, Tassinari MS, Stein GS, Lian JB. Factors that promote progressive development of the osteoblast phenotype in cultured fetal rat calvaria cells. *J Cell Physiol*. 1990;143(2):213–221.
28. Zhang Y, Ni M, Zhang M, Ratner B. Calcium phosphate-chitosan composite scaffolds for bone tissue engineering. *Tissue Eng*. 2003;9(2):337–345.
29. Mukherjee DP, Tunkle AS, Roberts RA, Clavenna A, Rogers S, Smith D. An animal evaluation of a paste of chitosan glutamate and hydroxyapatite as a synthetic bone graft material. *J Biomed Mater Res B Appl Biomater*. 2003;67(1):603–609.
30. Costa-Pinto AR, et al. Chitosan-poly(butylene succinate) scaffolds and human bone marrow stromal cells induce bone repair in a mouse calvaria model. *J Tissue Eng Regen Med*. 2012;6(1):21–28.
31. Hoemann CD, et al. Cytocompatible gel formation of chitosan-glycerol phosphate solutions supplemented with hydroxyl ethyl cellulose is due to the presence of glyoxal. *J Biomed Mater Res A*. 2007;83(2):521–529.
32. Bi L, Cheng W, Fan H, Pei G. Reconstruction of goat tibial defects using an injectable tricalcium phosphate/chitosan in combination with autologous platelet-rich plasma. *Biomaterials*. 2010;31(12):3201–3211.
33. Qu Z, Yan J, Li B, Zhuang J, Huang Y. Improving bone marrow stromal cell attachment on chitosan/hydroxyapatite scaffolds by an immobilized RGD peptide. *Biomed Mater*. 2010;5(6):065001.
34. Nandi SK, Kundu B, Basu D. Protein growth factors loaded highly porous chitosan scaffold: a comparison of bone healing properties. *Mater Sci Eng C Mater Biol Appl*. 2013;33(3):1267–1275.
35. Kawakami T, Antoh M, Hasegawa H, Yamagishi T, Ito M, Eda S. Experimental study on osteoconductive properties of a chitosan-bonded hydroxyapatite self-hardening paste. *Biomaterials*. 1992;13(11):759–763.

Synthetic grafts in the treatment of ruptured anterior cruciate ligament of the knee joint

Wojciech Satora^{1,A–F}, Aleksandra Królikowska^{2,A,E,F}, Andrzej Czamara^{2,A,E,F}, Paweł Reichert^{3,A,E,F}

¹ Department of Trauma and Orthopedic Surgery, St. Luke's Hospital, Bielsko-Biała, Poland

² College of Physiotherapy in Wrocław, Wrocław, Poland

³ Division of Sports Medicine, Wrocław Medical University, Wrocław, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;

D – writing the article; E – critical revision of the article; F – final approval of article

Polymers in Medicine, ISSN 0370-0747 (print), ISSN 2451-2699 (online)

Polim Med. 2017;47(1):55–59

Address for correspondence

Paweł Reichert

E-mail: pawelreichert74@gmail.com

Funding sources

none declared

Conflict of interest

none declared

Received on August 18, 2017

Revised on September 03, 2017

Accepted on September 09, 2017

Abstract

The anterior cruciate ligament (ACL) is cited as the most frequently injured ligament in the knee. The standard treatment of ACL injury remains ligament reconstruction followed by a postoperative physiotherapeutic procedure. During the reconstruction, the torn ligament can be replaced with an autograft or an allograft. A synthetic ligament is also one of the available graft options. Synthetic grafts in ruptured ACL treatment have been used as scaffolds, stents, or prostheses. The story of using synthetic materials in ACL deficient knee treatment started in the beginning of the 20th century with the usage of silk and silver fibers. The second half of the 20th century abounded in new synthetic materials being proposed as torn ACL replacements, such as Supramid®, Teflon® or Dacron®, Proplast®, carbon fiber graft, ABC graft, Kennedy-LAD®, Trevia, Leeds-Keio, Gore-Tex®, PDS®, EULIT®, and Polyflex® or LARS®. Artificial ligaments have intrigued surgeons for all these years as they represent the hope for grafts that are easily available and stronger than soft tissue “off-the-shelf” grafts, simplifying the surgery, and avoiding graft harvesting and donor site morbidity. However, most of the artificial grafts have been characterized by high rates of failure. One of the very few synthetic grafts gaining more widespread popularity has been LARS®. However, it is suggested that the ligament not be considered as a potential graft for primary reconstruction of the ACL, and it should be rather treated as an alternative graft in special cases, so the optimal synthetic graft material remains controversial.

Key words: artificial ligament, ligament prostheses, ligament reconstruction

DOI

10.17219/pim/76819

Copyright

© 2017 by Wrocław Medical University

This is an article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

The anterior cruciate ligament (ACL) is cited as the most frequently injured ligament in the human knee.^{1,2} In the USA, the average incidence frequency of ACL injuries amounts to 200,000 cases per year.³ The injuries most commonly occur in physically active individuals while playing multidirectional sports.¹ For patients wishing to return to sports activities, the standard treatment for ACL injury is the reconstruction of the ligament,⁴ followed by a postoperative physiotherapeutic procedure.⁵ The aim of the reconstruction is reinstating functional knee stability, and in turn, reducing the risk of secondary injuries, such as further damage to the menisci and degenerative osteoarthritis.^{6,7} A recent analysis of epidemiological data shows an increase of the incidence of ACL ruptures and subsequent reconstructions.⁸

Anterior cruciate ligament (ACL) of the knee joint

The ACL is one of the intra-articular ligaments of the knee joint. The ACL and the posterior cruciate ligament (PCL) are the most important stabilizers in the sagittal plane of the knee as they aim to stabilize the joint against the large anterior-posterior shear forces occurring while walking or running. “Cruciate”, literally meaning “cross”, and describes the shape of the ACL and PCL as they interconnect the tibia with the femur.

The ACL attaches to a facet on the anterior part of the intercondylar area of the tibia and ascends posteriorly where it is attached to a facet at the back of the lateral wall of the intercondylar fossa of the femur. Functionally, the ACL consists of 2 distinct bundles that are characterized by a spatial relationship throughout the knee flexion: the anteromedial (AM) and posterolateral (PL) bundles. The bundles also play different roles in the biomechanics and stability of the joint.^{9–11} Some authors have distinguished a 3rd bundle in the anatomy of the ACL, the intermediate (IM) bundle.^{12,13}

The ACL aims to resist anterior displacement and excessive rotation of the tibia relative to the femur.¹¹

ACL injury and its treatment

The ACL injury mechanism involves a combination of large rotational, side-to-side, and hyperextension forces through the knee.

During the reconstruction, the torn ligament can be replaced with an autograft or an allograft. Synthetic ligament usage is also one of the available graft options. Autograft choices involve the patellar, hamstring, and quadriceps tendons, while the allograft options consist of the quadriceps, patellar, Achilles, hamstring, and anterior and posterior tibialis tendons, and the fascia lata.¹⁴

Shorter surgical and anesthesia time, fewer postoperative complications, reduced morbidity at the harvest site, faster postoperative recovery and lower incidence of postoperative arthrofibrosis, and less postoperative pain are considered to be the main advantages of allograft usage for ACL replacement.^{15,16} On the other hand, allograft usage may be associated with higher rates of re-rupture, limited availability, a delayed healing and ligamentization process in comparison to autografts, risk of disease transmission and expensiveness.^{17–19} The synthetic materials being used in ACL reconstruction were introduced with an aim to improve the strength and stability of the graft immediately after the reconstruction, reduce donor site morbidity and eliminate the potential for disease transmission.²⁰ However, the first artificial grafts were characterized by high rates of failure and synovitis reactivation,^{21,22} thus with advancing technology, new synthetic materials have been developed for ACL reconstruction grafts.²³ The optimal graft material remains controversial.

Artificial ligament usage in ACL reconstruction

Synthetic grafts in ruptured ACL treatment have been used as scaffolds, stents, or prostheses.¹⁴

A scaffold, such as the carbon fiber scaffold ligament, was introduced with the purpose of stimulating fibrous tissue ingrowth, and contributes to the ultimate strength of the new ligament.²⁴ An example of a synthetic stent is the Kennedy ligament augmentation device (LAD).²⁴ An example of a prosthetic graft was the Gore-Tex graft, Stryker Dacron graft, or ABC graft.

The first half of the 20th century

The history of the use of synthetic materials to replace the ACL goes back to the year 1903, when prosthetic ligaments made of silk sutures were introduced by Fritz Lange of Munich.²⁵ Four years later, Lange reported on 4 cases of patients with ACL deficiency whose knees were stabilized with the use of artificial ligaments made of silk in conjunction with the semitendinosus and semimembranosus muscle tendons placed extra-articularly.²⁶ However, in 1918, Smith of Cardiff criticized the silk fibers, considering them to be the cause of synovitis in his patient,²¹ sharing the same opinion with Max Herz (1906),²² and confirming the failure of silk usage in isolation within the intra-articular environment. The answer to that was introducing a foreign body scaffold aiming to provide initial strength during ligament healing and re-growth, and utilizing in ACL reconstruction silk augmented with fascia by Max Fritz of Munich,²⁷ followed by Karl Ludloff of Frankfurt in 1927.²⁸

Another example of a synthetic material being used for ACL replacement was a loop of silver wire introduced by Edred Corner of London in 1914.²⁹

The second half of the 20th century

In 1949, Ruther of Germany introduced a synthetic ligament for ACL replacement made of a polyamide derivative, and named Supramid.³⁰ Nevertheless, the results of treatment with the use of Supramid were disappointing.³⁰

After a successful implantation of Teflon grafts into dog knees,³¹ Olav Rostrup of Edmonton in 1959 started using the synthetic material in humans.³² He didn't recommend Teflon or Dacron for wide-scale use as he considered synthetic materials to be rather supporting devices, instead of ligament replacements. Even so, the ligament replacement device named Dacron was made commercially available in the 1980s by Stryker, but production was finally discontinued in 1994 as the results of treatment with its use were not satisfying. An evaluation carried out by Wilk and Richmond (1993) of patients after ACL reconstruction with the use of Dacron revealed significant deterioration of the ligament failure rate from 20% 2 years postoperatively up to 37.5% at 5 years.³³ The high rate of graft failure was confirmed by Maletius and Gillquist (1997) in a 9-year follow-up, amounting 44%.³⁴

Another synthetic graft being used in the second half of the 20th century was a porous Teflon graft, named Proplast. Interestingly, it was one of the first synthetic graft materials approved by the Food and Drug Administration (FDA), however, the results of its clinical usage were not satisfying.³⁵

Also, flexible carbon fiber was introduced as a synthetic graft material for ACL reconstruction.³⁶ David Jenkins of Cardiff started to use them in the mid-1980s as a scaffold aiming to encourage the ingrowth of fibroblastic tissue and production of new collagen. Nevertheless, the occurrence of the fragmentation of carbon creating unsightly staining of the synovium and foreign body reaction was noted. In 1985, Angus Strover of South Africa didn't notice any occurrence of carbon debris in the knee joint cavity when the carbon fibers were used with the remnants of the original ligament or within a fascia sheath.³⁷

When it comes to Poland, the wide-ranging studies concerning carbon fiber usage generally in knee surgery and knee ligament reconstructions, especially in ACL reconstruction, have been carried out mainly by Andrzej Górecki and Wojciech Kuś in the Clinics of Orthopedics and Traumatology of the Medical University in Warszawa in cooperation with the AGH University of Science and Technology in Kraków.^{38,39}

The mid-1980s also brought the ligament replacement named Activated Biological Composite (ABC). The ABC graft, being a combination of polyester and carbon fiber, was placed through anatomical bony tunnels. In the beginning, the ligament gained popularity, however, eventually became obsolete like other synthetic grafts.²³

An example of an ACL augmentation device was the Kennedy-LAD, made of polypropylene and introduced by Jack Kennedy of London in the late 1970s.⁴⁰ The synthetic

stent was sutured to an autologous graft and fixed to the bone at both ends, with the aim to support and protect the autologous graft during the healing phase, when the autologous tissue was the weakest. The LAD was a band-like braid of propylene that was designed to protect the autogenous graft from excessive stresses, however it happened to stress the autologous graft, leading to failure.⁴¹ One polyester graft resembling LAD in design, but being placed in a nonanatomic position was Trevia. The Leeds-Keio was a polyester mesh graft designed in order to augment the autogenous graft that was placed through bony tunnels and fixed outside the tunnel with staples. A Murray et al. study reporting the long-term results of patients 10–16 years after ACL reconstruction with the use of the Leeds-Keio ligament revealed rather poor results with a high rate of ligament rupture and knee laxity.⁴² Also, LAD usage results indicted a high rate of postoperative complications and re-surgeries,⁴³ as well as causing effusions and synovitis reactivation and autogenous graft delay.⁴⁴ All in all, it was suggested not to use Leeds-Keio or LAD devices due to their poor outcomes.⁴⁵

Other examples of synthetic grafts were Gore-Tex, PDS, EULIT, and Polyflex.^{23,46} The Gore-Tex graft was a prosthetic graft that, theoretically, aiming to avoid the bending forces at the entrance to the femoral tunnel, was placed in a nonanatomic position over the top of the femur.

Ligament advanced reinforcement system (LARS)

One of the synthetic material grafts introduced in the second half of the 20th century was a graft made of polyester named Ligostic, which evolved to a non-absorbable synthetic ligament device made of terephthalic polyethylene polyester fibers, the Ligament Advanced Reinforcement System (LARS).⁴⁷ The LARS is intensively cleaned with the aim to remove potential machining residues and oils to further encourage soft tissue in-growth and reduce the risk of reactive synovitis. The LARS consists of 2 parts, an intraarticular part and an extraarticular one. The ligament intra-articular portion/scaffold is built of multiple parallel fibers that are twisted at 90-degree angles.⁴⁷ The part is composed of 2 longitudinal external rotation fibers without transverse fibers, being designed as an imitation of ACL anatomic structure. The extraarticular part is waved by longitudinal and transverse fibers with the aim to avoid ligament deformation. The short-term postoperative results of patients after ACL reconstruction with the use of LARS are very satisfying.⁴⁸ A mean 2.5-year follow up carried out by Dericks in 1995 revealed encouraging results of treatment with the use of LARS.⁴⁷ Also, a follow-up at a mean of 8 years reported by Parchi et al. in 2013 showed satisfying results reflected in no postoperative complication occurrence and only one case of LARS rupture.⁴⁹ On the other hand, the follow-up of 10 years postoperatively carried out by Tiefenboeck et al. (2015)

revealed lack of subjective satisfaction in half of patients treated with the use of LARS, thus the authors suggested not to consider the ligament system as a potential graft for primary reconstruction of the ACL and rather treat it as an alternative graft in special cases.⁵⁰

Summary

The story of using synthetic materials in ACL deficient knee treatment started in the beginning of 20th century with the usage of silk and silver fibers. The story of using synthetic materials in ACL deficient knee treatment started in the beginning of the 20th century with the usage of silk and silver fibers. The second half of the 20th century abounded in new synthetic materials being proposed as torn ACL replacements such as Supramid, Teflon or Dacron, Proplast, carbon fiber graft, ABC graft, Kennedy-LAD, Trevia, Leeds-Keio, Gore-Tex, PDS, EULIT, and Polyflex or LARS.

Artificial ligaments have intrigued surgeons for all these years as they represent the hope for grafts that are easily available and stronger than soft tissue “off-the-shelf” grafts, simplifying the surgery, and avoiding graft harvesting and donor site morbidity. However, most of the artificial grafts have been characterized by high rates of failures. One of the very few synthetic grafts gaining more widespread popularity and which remains in use as an augmentation device up to this day has been LARS. However, it is suggested that the ligament should not be considered as a potential graft for primary reconstruction of the ACL, but should be rather treated as an alternative graft in special cases, so the optimal synthetic graft material remains controversial.

References

- Gianotti SM, et al. Incidence of anterior cruciate ligament injury and other knee ligament injuries: A national population-based study. *J Sci Med Sport*. 2009;12(6):622–627.
- Janssen KW, et al. High incidence and costs for anterior cruciate ligament reconstructions performed in Australia from 2003–2004 to 2007–2008: Time for an anterior cruciate ligament register by Scandinavian model? *Scand J Med Sci Sports*. 2012;22(4):495–501.
- Prodromos CC, et al. A meta-analysis of the incidence of anterior cruciate ligament tears as a function of gender, sport, and a knee injury-reduction regimen. *Arthroscopy*. 2007;23(12):1320–1325.
- Ekstrand J. A 94% return to elite level football after ACL surgery: A proof of possibilities with optimal caretaking or a sign of knee abuse? *Knee Surg, Sports Traumatol, Arthrosc*. 2011;19(1):1–2.
- Arna Risberg M, Lewek M, Snyder-Mackler L. A systematic review of evidence for anterior cruciate ligament rehabilitation: how much and what type? *Phys Ther Sport*. 2004;5(3):125–145.
- George MS, Dunn WR, Spindler KP. Current concepts review: Revision anterior cruciate ligament reconstruction. *Am J Sports Med*. 2006;34(12):2026–2037.
- Chaudhari AM, et al. Knee kinematics, cartilage morphology, and osteoarthritis after ACL injury. *Med Sci Sports Exerc*. 2008;40(2):215–222.
- Buller LT, et al. Trends in anterior cruciate ligament reconstruction in the United States. *Orthop J Sports Med*. 2015;3(1):23259671–14563664.
- Palmer I. On the injuries to the ligaments of the knee joint: A clinical study. *Acta Chir Scand*. 1938;91:282.
- Abbott LC, et al. Injuries to the ligaments of the knee joint. *J Bone Joint Surg*. 1944;26(3):18.
- Girgis FG, Marshall JL, Monajem A. The cruciate ligaments of the knee joint. Anatomical, functional and experimental analysis. *Clin Orthop Relat Res*. 1975(106):216–231.
- Norwood LA, Cross MJ. Anterior cruciate ligament: Functional anatomy of its bundles in rotatory instabilities. *Am J Sports Med*. 1979;7(1):23–26.
- Amis AA, Dawkins GP. Functional anatomy of the anterior cruciate ligament. Fibre bundle actions related to ligament replacements and injuries. *J Bone Joint Surg Br*. 1991;73(2):260–267.
- West RV, Harner CD. Graft selection in anterior cruciate ligament reconstruction. *J Am Acad Orthop Surg*. 2005;13(3):197–207.
- Cohen SB, Sekiya JK. Allograft safety in anterior cruciate ligament reconstruction. *Clin Sports Med*. 2007;26(4):597–605.
- Zeng C, et al. Autograft versus allograft in anterior cruciate ligament reconstruction: A meta-analysis of randomized controlled trials and systematic review of overlapping systematic reviews. *Arthroscopy*. 2016;32(1):153–163.
- Samuelsson K, et al. Trends in surgeon preferences on anterior cruciate ligament reconstructive techniques. *Clin Sports Med*. 2013;32(1):111–126.
- Barrera Oro F, et al. Autograft versus allograft: An economic cost comparison of anterior cruciate ligament reconstruction. *Arthroscopy*. 2011;27(9):1219–1225.
- Cooper MT, Kaeding C. Comparison of the hospital cost of autograft versus allograft soft-tissue anterior cruciate ligament reconstructions. *Arthroscopy*. 2010;26(11):1478–1482.
- Johnson D. Why synthetic grafts failed, in the anterior cruciate ligament: Reconstruction and basic science. C. Prodromos, C., Editor. 2008, Saunders Elsevier: Philadelphia, 88–90.
- Smith A. The diagnosis and treatment of injuries to the crucial ligaments. *Br J Surg*. 1918;6:176–189.
- Herz M. Die chirurgische Behandlung paralytischer Schlotterelenke-Seitenligamente oder Arthrodesen? [The surgical treatment of paralytic flail knees: Silk ligaments or arthrodesis]. *Munch Med Wschr*. 1906;51:2527–2529.
- Schindler OS. Surgery for anterior cruciate ligament deficiency: A historical perspective. *Knee Surg Sports Traumatol Arthrosc*. 2012;20(1):5–47.
- Zoltan DJ, Reinecke C, Indelicato PA. Synthetic and allograft anterior cruciate ligament reconstruction. *Clin Sports Med*. 1988;7(4):773–784.
- Lange F. Über the Sehnenplastik [On the tendon reconstruction]. *Verh Dtsch Orthop Ges*. 1903;2:10–12.
- Lange F. Kunstliche Gelenkbänder aus Seide [On artificial silk ligaments]. *Munch Med Wschr*. 1907;52:834–836.
- Lange M. Unfallorthopädie. 1949, Stuttgart: Ferdinand Enke.
- Ludloff K. Der operative Ersatz des vorderen Kreuzbandes am Knie [Surgical replacement of the anterior cruciate ligament]. *Zentralbl Chir*. 1927;54:3162–3166.
- Corner EM. The exploration of the knee-joint: With some illustrative cases. *Br J Surg*. 1914;2(6):191–204.
- Ruther H. Die Verwendung von Supramid in der operativen Orthopädie. *Z Orthop*. 1949;78:161–169.
- Emery MA, Rostrup O. Repair of the anterior cruciate ligament with 8 mm, tube teflon in dogs. *Can J Surg*. 1960;4:111–115.
- Rostrup O. Reconstruction of the anterior cruciate ligaments. *West J Surg Obstet Gynecol*. 1964;72:199–202.
- Wilk RM, Richmond JC. Dacron ligament reconstruction for chronic anterior cruciate ligament insufficiency. *Am J Sports Med*. 1993;21(3):374–379.
- Maletius W, Gillquist J. Long-term results of anterior cruciate ligament reconstruction with a Dacron prosthesis. The frequency of osteoarthritis after seven to eleven years. *Am J Sports Med*. 1997;25(3):288–293.
- Woods GW, et al. Proplast leader for use in cruciate ligament reconstruction. *Am J Sports Med*. 1979;7(6):314–320.
- Jenkins DH. The repair of cruciate ligaments with flexible carbon fibre. A longer term study of the induction of new ligaments and of the fate of the implanted carbon. *J Bone Joint Surg Br*. 1978;60-b(4):520–522.
- Strover AE, Firer P. The use of carbon fiber implants in anterior cruciate ligament surgery. *Clin Orthop Relat Res*. 1985(196):88–98.
- Górecki A. Przydatność włókien węglowych w śródstawowych rekon-

- strukcjach przedniego więzadła krzyżowego stawu kolanowego. 1983, Rozprawa doktorska, Akademia Medyczna w Warszawie.
39. Kuś W. Badania doświadczalne nad zastosowaniem włókien węglowych w operacjach odtwórczych narządu ruchu. 1986, Rozprawa habilitacyjna, Akademia Medyczna w Warszawie.
 40. Kennedy J, Willis R. Synthetic cruciate ligaments: Preliminary report. *J Bone Jt Surg*. 1976;58(B):142.
 41. Drogset JO, et al. A sixteen-year follow-up of three operative techniques for the treatment of acute ruptures of the anterior cruciate ligament. *J Bone Joint Surg Am*. 2006;88(5):944–952.
 42. Murray AW, Macnicol MF. 10–16 year results of Leeds-Keio anterior cruciate ligament reconstruction. *The Knee*. 2004;11(1):9–14.
 43. Riel KA. Augmented anterior cruciate ligament replacement with the Kennedy-LAD (ligament augmentation device) – long term outcome. *Zentralbl Chir*. 1998;123(9):1014–1018.
 44. Kumar K, Maffulli N. The ligament augmentation device: A historical perspective. *Arthroscopy*. 1999;15(4):422–432.
 45. Jia ZY, et al. Comparison of artificial graft versus autograft in anterior cruciate ligament reconstruction: A meta-analysis. *BMC Musculoskelet Disord*. 2017;18(1):309.
 46. James SL, et al. Cruciate ligament stents in reconstruction of the unstable knee. A preliminary report. *Clin Orthop Relat Res*. 1979(143):90–96.
 47. Dericks G. Ligament advanced reinforcement system anterior cruciate ligament reconstruction. *Operative Techniques in Sports Medicine*. 1995;3(3):187–205.
 48. Krupa S, Krolikowska A, Reichert P. Postoperative knee joint stability following anterior cruciate ligament reconstruction using the ligament advanced reinforcement system. *Polim Med*. 2016;46(2):155–161.
 49. Parchi PD, et al. Anterior cruciate ligament reconstruction with LARS artificial ligament results at a mean follow-up of eight years. *Int Orthop*. 2013;37(8):1567–1574.
 50. Tiefenboeck TM, et al. Clinical and functional outcome after anterior cruciate ligament reconstruction using the LARS system at a minimum follow-up of 10 years. *Knee*. 2015;22(6):565–568.

Polimery w Medycynie
Polymers in Medicine

