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Innovative platforms for oral microencapsulation formulation and drug analytical techniques for anti-diabetics

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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

1st September 2020

Acknowledgment

All praises and thanks due to ‘’Allah’’, the most beneficent, the most merciful, the most gracious to shower me with all his endless blessings including the achievement of this Master of Philosophy in Pharmacy.

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Abstract

Metformin, first discovered in the 1950's from herbs, became the first line anti-diabetic agent in many countries. This interesting biguanide has various other physiological roles apart from its glycemic one. One of the major limitations that clinicians face in metformin-based therapy lies in its inherent pharmacokinetic properties, where the biguanide is eliminated from the body at a rate that is faster than its absorption rate (flip-flop kinetics) with a short biological half-life.

Metformin is also classified by the Biopharmaceutics Classification System and Biopharmaceutics Drug Disposition Classification System as a Class III therapeutic with limited oral bioavailability. Of importance, microencapsulation is an acceptable approach used to overcome the pharmacokinetic limitations of metformin, and which could improve its oral bioavailability. Robust and reproducible techniques for metformin analysis on the other hand are quite challenging due to its polar nature, small molecular size, and its hygroscopic nature. These challenges to analyse, detect or quantify metformin are more pronounced when metformin is combined with other therapeutics in the same dosage form or in biological samples.

The first objective of this project was to develop different formulations of silicon-based metformin microcapsules produced by vibrational jet flow ionotropic gelation technique. Full characterisation experiments of the developed microcapsules were conducted in terms of rheological properties, electro-kinetic stability, surface tension, drug loading, encapsulation efficiency, mechanical strength, swelling characteristics and microscopic examination.

The project also aimed to develop and validate a new analytical method to detect and quantify metformin alone and when combined with another therapeutic agent (gliclazide) that belongs to a class of antidiabetic agents called sulfonylureas. This new method was compliant with the International Conference of Harmonisation (Geneva) and was applied to analyse marketed pharmaceutical tablets of metformin and gliclazide.

The last objective of this project was to undergo multifaceted forced degradation studies on metformin, gliclazide and another sulfonylurea (glipizide) by applying this new analytical method developed in my project to verify its suitability as a stability indicating tool for the analysis of these three antidiabetic agents either for research purposes or in pharmaceutical industry application.

List of publications included as a part of the thesis

- **Gedawy A**, Al-Salami H, Dass CR. Role of metformin in various pathologies: state-of-the-art microcapsules for improving its pharmacokinetics. Therapeutic Delivery. 2020, *Published online/ahead of print as of 23rd of November 2020.*

- **Gedawy A**, Dass CR, Al-Salami H. Polydimethylsiloxane-customized nanoplatform for delivery of antidiabetic drugs. Therapeutic Delivery. 2020;11(7):415-429.

- **Gedawy A**, Al-Salami H, Dass CR. Advanced and multifaceted stability profiling of the first-line antidiabetic drugs metformin, gliclazide and glipizide under various controlled stress conditions. Saudi Pharmaceutical Journal. 2020;28(3):362-368.

- **Gedawy A**, Al-Salami H, Dass CR. Development and validation of a new analytical HPLC method for simultaneous determination of the antidiabetic drugs, metformin and gliclazide. Journal of Food and Drug Analysis. 2019;27(1):315-322.

Relevant publication to the thesis but not forming a part of it

- **Gedawy A**, Martinez J, Al-Salami H, Dass CR. Oral insulin delivery: existing barriers and current counter-strategies. Journal of Pharmacy and Pharmacology. 2018;70(2):197-213.

Conference poster

- **Gedawy A**, Al-Salami H, Dass CR. Novel analytical method for simultaneous determination of the binary mixture of antidiabetic drugs, metformin and gliclazide.

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Statement of contribution of others

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Abbreviations

ADA	American Diabetes Association
AMP	Adenosine monophosphate
AMPK	Adenosine monophosphate activated protein kinase
ATP	Adenosine triphosphate
BCS	Biopharmaceutics Classification System
BDDCS	Biopharmaceutics Drug Disposition Classification System
$CaCl_2$	Calcium chloride
CHF	Congestive heart failure
COVID-19	2019 novel coronavirus disease
cps	Centipoise
CRP	C-reactive protein
DNA	Deoxyribonucleic acid
EASD	European Association for the Study of Diabetes
EDS	Energy dispersive x-ray
eGFR	Estimated glomerular filtration rate
EM	Electron microscopy
FDA	Food and Drug Administration
FTIR	Fourier transform infrared
GIT	Gastrointestinal tract
GLUT2	Glucose transporter 2
GLUT4	Glucose transporter 4
H_2O_2	Hydrogen peroxide
HbA1c	Glycated haemoglobin
HCl	Hydrochloric acid
HER-2	Human epidermal growth factor receptor 2
HIV	Human immunodeficiency virus
HPLC	High performance liquid chromatography
HPMC	Hydroxy propyl methyl cellulose
ICH	International Conference of Harmonization
IGF-1	Insulin-like growth factor 1
LC/MS	Liquid chromatography/ mass spectroscopy
LDL	Low-density lipoprotein
LKB1	Liver kinase B1
LOD	Limit of detection
LOQ	Limit of quantification
MATE	Multidrug and toxin extrusion transporter
mPa.s	Millipascal-second
mTOR	Mammalian target of rapamycin

NaCl	Sodium chloride
NAD	Nicotinamide adenine dinucleotide
NaOH	Sodium hydroxide
NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B cells
OCTN1	Carnitine/organic cation transporter
OCTs	Organic cation transporters
PAI-1	Plasminogen activator inhibitor 1
PCOS	Polycystic ovary syndrome
PCP	Polycarbophil
PDMS	Polydimethylsiloxane
PI3K	Phosphatidylinositol-3-kinase
PKA	cAMP-dependent protein kinase
PMAT	Plasma membrane monoamine transporter
PVP	Polyvinylpyrrolidone
RH	Relative humidity
SECS	Salivary Excretion Classification System
SERT	Serotonin reuptake transporter
sICAM-1	Soluble intercellular adhesion molecule 1
sVCAM-1	Soluble vascular adhesion molecule 1
T2D	Type 2 diabetes
TF	Tissue factor
THTR-2	Thiamine transporter
TNF	Tissue necrosis factor
UCP2	Uncoupled protein 2
UKPDS	United Kingdom Prospective Diabetes Study
vWF	von Willebrand factor
WHO	World Health Organization

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Chapter 1

Graphical abstract

Chapter 1: Role of metformin in various pathologies: state-of-the-art microcapsules for improving its pharmacokinetics

Gedawy A et al. 2020. “Role of metformin in various pathologies: state-of-the-art microcapsules for improving its pharmacokinetics”

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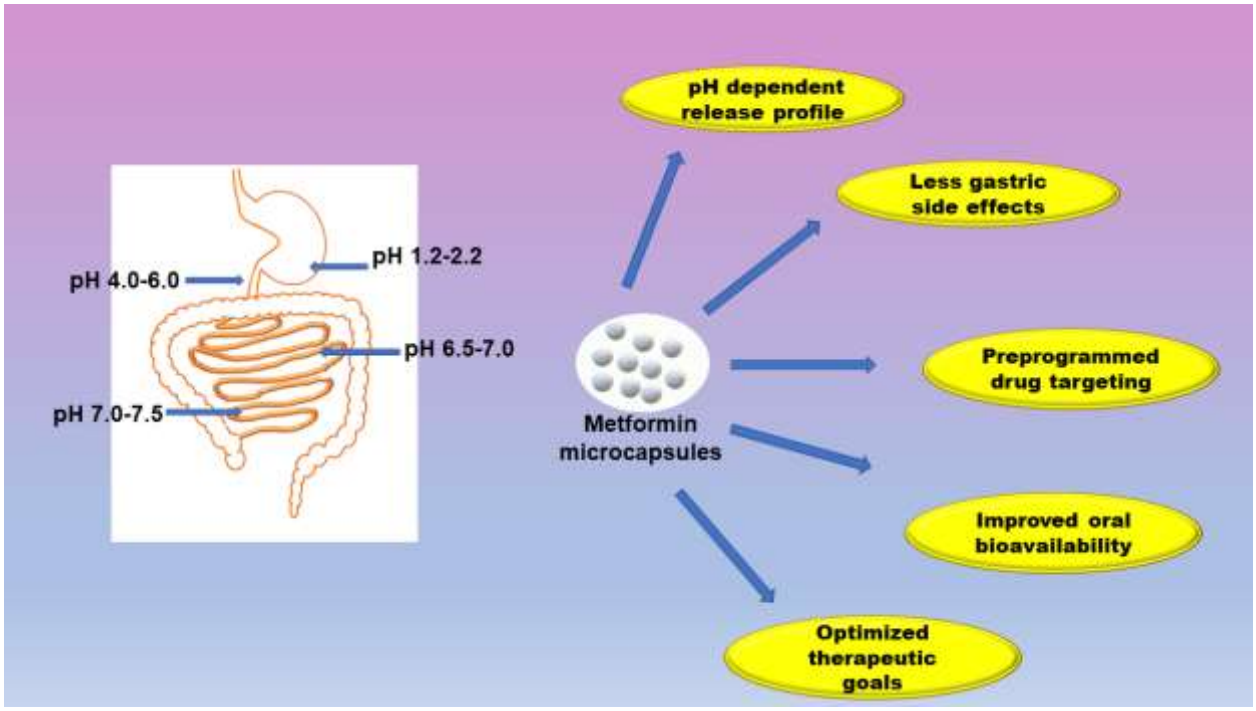

This chapter is introductory to this thesis that outlines the pharmacodynamics of metformin and its pharmacokinetics including major transporters involved in absorption, distribution and elimination of metformin in addition to the *status quo* and recent advances in microencapsulation of this biguanide.

Abstract

Background: Metformin that was originally derived from botanical ancestry, became the most prescribed, first line therapy for type 2 diabetes in most countries. In the last century, metformin was discovered twice for its anti-glycemic properties in addition to its antimalarial and anti-influenza effects. Metformin exhibits flip-flop pharmacokinetics with limited oral bioavailability.

Objective: This review outlines metformin pharmacokinetics, pharmacodynamics and recent advances in polymeric particulate delivery systems as a potential tool to target metformin delivery to specific tissues/organs.

Conclusion: This interesting biguanide is being rediscovered this century for multiple clinical indications as anticancer, antiaging, anti-inflammatory, anti-Alzheimer's and much more. Microparticulate delivery systems of metformin may improve its oral bioavailability and optimize the therapeutic goals expected.

1. Introduction

In 2019, 463 million diabetic patients were estimated globally [1], while 379 million were estimated globally to suffer impaired glucose tolerance [1]. These figures are expected to grow to 700 million diabetic patients and 548 million prediabetic subjects by 2045 [1] . The World Health Organization (WHO) is predicting diabetes to constitute the seventh major cause of deaths in 2030 [2]. The majority of diabetic population (90%) suffer from type 2 diabetes (T2D) [1], which could be defined as a pandemic metabolic disorder of glucose homeostasis and impaired lipid metabolism, where these metabolic disorders could potentially lead to fatal cardiovascular events [3, 4].

T2D is sometimes called insulin resistance syndrome or metabolic syndrome, reported by Reaven as Syndrome X [5]. T2D could also be referred to as the silent killer, where the disease can develop and progress before it gets diagnosed due to lack of the classical diabetic symptoms of polydipsia and polyuria [2, 3, 6]. Peripheral insulin resistance and inadequate β-cell function are the main pathophysiological features of T2D, where the regulatory pathway of insulin secretion and insulin sensitivity is impaired and manifested as excessive hepatic glucose production and reduced glucose uptake by muscle and adipose tissue [4, 7-9].

Imbalanced diet and sedentary lifestyle were reported to cause impaired glucose tolerance and impaired fasting glucose (prediabetes), objects with these conditions are more prone to T2D that can be developed gradually over few years [2, 9]. Healthy lifestyle, balanced diet and exercise could reverse prediabetic conditions and decrease incidence of diabetes by 58% [4, 7]. Obesity, visceral adiposity and intraabdominal fat are major determinants for insulin resistance. Of note, obese adolescents with insulin resistance can develop T2D quicker than adults [4, 7, 8].

Insulin resistance is considered the main predictor of T2D as it could happen few years before the diagnosis of T2D and the mechanism by which it deteriorates the β-cell function is still unclear [8]. Glycated hemoglobin (HbA1c of < 7%) is the glycemic target set for T2D patients to delay the progression of microvascular complications, where up to 75% reduction in diabetic neuropathy, diabetic retinopathy and diabetic nephropathy were reported at this glycemic level [10].

First line therapy of T2D as reported by the European Association for the Study of Diabetes (EASD) and the American Diabetes Association (ADA) in their 2019 updates, is metformin and comprehensive lifestyle intervention of physical activity and weight management [11-13]. Metformin was reported to reduce the rate of diabetes progression by around 30% compared to placebo [7]. The antihyperglycemic effect of metformin is

predominantly achieved by restoring the peripheral tissue and hepatic sensitivity to insulin without interfering with its endogenous secretion [14].

Metformin also delayed or prevented the incidence of T2D in various clinical trials [15]. The risk of diabetic retinopathy (non-proliferative type) was remarkably reduced in diabetic patients on metformin [16]. Unlike other biguanides that were withdrawn from the market due to their high incidence of lactic acidosis [17], metformin-associated lactic acidosis is very rare (in 100000 object-years, less than 10 incidences were reported) [18]. No risk of lactic acidosis was reported with metformin therapy in advanced chronic kidney disease [19].

Metformin is often called the aspirin of this century [20], where it gained special importance in many non-diabetic conditions such as cancer [21], Alzheimer's [22], inflammation [23] and aging [24]. Surprisingly, due to its multiple unique cellular pathways, metformin was proposed to be investigated in the treatment of the pandemic (COVID-19) virus [25] relying on its reported antiviral potential [26, 27]. The pharmacokinetics of metformin seem to be problematic for most clinicians, where a repeated administration of high doses are often needed to achieve therapeutic targets [14, 28-30].

This review demonstrates metformin pharmacokinetics, pharmacodynamics, versatile polymeric platforms and recent approaches in polymeric microparticulate metformin delivery that might help to improve the bioavailability of the biguanide.

2. Metformin chemistry and physicochemical properties

Metformin (1,1-dimethylbiguanide) (molecular mass, 129.16 g/mol) is a planar small molecule with non-polar methyl terminals [17, 31, 32]. Metformin has a single protonation site between two imino groups which possess copper-chelating properties [17]. The intermolecular hydrogen bonds are responsible for the stability of metformin crystal structure [17]. Metformin exists as hydrochloride salt in the form of white, hygroscopic crystals (molecular mass. 165.6 g/mol) [33].

The polar biguanide has a pKa of 2.8 and 11.5 [34] with a melting point ranges from 223^{o}C to 226^{o}C. At physiological pH, metformin is ionized and exists as a hydrophilic and positively-charged species[14, 17, 35]. These characteristics accounts for classifying metformin by Biopharmaceutics Drug Disposition Classification System (BDDCS) and Biopharmaceutics Classification System (BCS) as Class III substance with high solubility, low membrane permeability and poor metabolism [35-38]. Metformin is also classified by Salivary Excretion Classification System (SECS) as Class II material with

low protein binding and low membrane permeability which allows its excretion in saliva [39].

3. Metformin origin and history

Metformin's background dates back to 1772 when John Hill used *Galega Officinalis* to correct the symptoms of polydipsia and polyurea associated with diabetes [17, 40]. The traditional medicine *Galega Officinalis* was known in medieval Europe under many names (Professor weed, Italian fitch, Goat's rue, Spanish sainfoin or French lilac) [17]. The phytochemistry of the herb revealed that *Galega Officinalis* is rich in guanidine which was later in 1918 reported to induce hypoglycemia in the animal model [17, 41]. Metformin belongs to a class chemically known as biguanides that were proved in 1929 to have antihyperglycemic effect in animals [17, 42, 43].

Interestingly two decades later in Philippines, metformin was rediscovered as antimalarial and anti-influenza [17]. Eusebio Garcia tested metformin for its antimalarial potential after its chemical conversion from the antimalarial agent (proguanil) and metformin was also used against local influenza under the name of (flumamine) with noticeable decrease in blood glucose level [17, 44]. The pharmacotherapeutics of metformin were first published in 1957 by Jean Sterne [17, 45].

Metformin was then marketed under the name (Glucophage®), a name that was suggested by Sterne and means the 'glucose eater' [17]. One year later, metformin was introduced in Europe as a diabetic agent [17], however it was not until 1972 that metformin was made available in Canada [17]. The Food and Drug Administration (FDA) approved metformin and it was introduced in the US market by 1995 [17]. The long-term cardiovascular benefits and metabolic effects was documented in the UK prospective diabetes study (UKPDS) in 1998 [17]. Metformin was enlisted in the essential medicines by WHO in 2011 [17].

4. Pharmacokinetics of metformin

After its oral administration, metformin exhibits incomplete and slow absorption from the gastrointestinal tract (GIT) [14, 28]. Negligible absorption of metformin from stomach (10% over 4 hours) and large intestine were reported, however the absorption process is chiefly accomplished at proximal small intestine (duodenum and jejunum)

[14, 28, 46]. The absolute oral bioavailability following ingestion of 500 mg immediate release metformin formula is low (40-60%) [14, 28, 46]. Within 6-10 hours of oral ingestion of metformin, the absorption process ceases regardless of the metformin dose [14, 28]. Long linear pharmacokinetics of metformin were described in diabetic and non-diabetic objects for doses up to 1500 mg [14]. Metformin reaches peak plasma concentration (C_{max}) ranges from 1-1.6 mg/L and up to 3 mg/L within 3 hours after oral administration of doses 500mg and 1500 mg respectively [28].

The rate of metformin absorption and its bioavailability is remarkably decreased with higher metformin doses than small doses [45] in an indication of an inverse relationship between metformin dose and the corresponding pharmacokinetic parameters [14, 28] where, the absorption from 500mg dose recorded higher bioavailability than that from 850 mg and 1500 mg [14] doses. A rapid considerable tissue uptake of metformin takes place following its absorption without any evidence of plasma protein binding which contribute to the high volume of metformin distribution (*Vd*, 63 to 276 L) [14, 28].

Metformin accumulation in salivary glands, enterocytes, erythrocytes, oesophagus and kidneys was also reported [14, 28] [45]. 20-30% of metformin can be recovered in faeces after oral administration however no faecal recovery of metformin following intravenous dose [14, 28, 30]. Metformin does not undergo hepatic metabolism with no conjugates identified [14, 28, 30].

Rapid renal excretion of metformin is the predominant mode of metformin clearance where 30-50% of metformin dose is eliminated unchanged in urine [14, 28, 30]. Dose adjustment is required for metformin patients with impaired renal function [28]. Metformin was reported to have (flip-flop pharmacokinetics) characterized by faster rate of metformin plasma elimination than its oral absorption [14, 28, 37] [45].

Clinicians can safely recommend metformin in gestational diabetes where pregnant women experience lower metformin plasma concentration than non-pregnant women due to the high glomerular filtration rate and metformin could cross the placenta and reaches the fetus at much lower concentrations than the mother's [28, 30]. Low metformin concentrations are detected in the breast milk of lactating mothers which are considered safe for the infants [28, 31].

Passive diffusion of metformin across the cell membrane is unlikely to happen as it is ionized (cationic) at physiological pH and due to its hydrophilic nature, however the remarkable tissue uptake and the large volume of distribution suggest that metformin is subjected to different types of drug transporters that govern its absorption, distribution and elimination [14, 28, 38]. **Table 1** summarizes major transporters involved in metformin clearance, distribution and absorption.

4.1. Metformin absorption

Gastrointestinal absorption and hepatic metabolism are generally considered the main contributing parameters in drug bioavailability. Absence of metformin's hepatic metabolism would suggest that metformin's absorption from GIT is the predominant rate limiting step in its pharmacokinetics and the overall bioavailability[38, 45]. While metformin's absorption is hindered by higher ingested doses, a saturable active process is suggested for its absorption [38]. In 2004, plasma membrane monoamine transporter (PMAT) was identified and is deemed responsible for the intestinal transport of several hydrophilic cationic substrates such as metformin [38].

Metformin's uptake by enterocytes can be mediated by organic cationic transporters (OCT1 and OCT3) expressed to a lesser extent in GIT, yet with a pivotal role where metformin's absorption was dramatically dropped in OCT3 deficient mice [28, 38]. Intestinal absorption of metformin can also be mediated by carnitine/organic cation transporter (OCTN1) [38]. In vitro, serotonin reuptake transporter (SERT) was reported to facilitate metformin's transport in Caco-2 cell lines [38]. Thiamin transporter (THTR-2), a pH-sensitive transporter was reported to transport metformin, where improved metformin uptake was observed at low pH [38].

4.2. Metformin distribution

OCT1 and OCT3 are deemed the major transporters in hepatic uptake of metformin [28, 38]. In OCT1 knockout mice, not only a significant reduction in hepatic metformin concentration was reported but the antihyperglycemic effect of metformin was totally vanished [29, 30, 38]. There was a belief that the metformin caused reduction in glycated haemoglobin (Hb1Ac) is dependent on OCT1 activity [30]. Tissue uptake and metformin distribution could be attributed to OCT3 that is expressed in various organs/tissues, where the volume of distribution of metformin is almost halved in OCT3-deficient mice [38].

On the other hand, glucose transporter (GLUT4) expression in the adipose tissue of these mice was remarkably decrease compared to wild type mice [38]. Metformin transport into bile canaliculi could be facilitated by multidrug and toxin extrusion transporter (MATE-1) [28]. The uptake of metformin by the skeletal muscles was reported to be mediated predominantly by OCT3 and OCT1 to a lesser extent [28]. Taste disturbance associated with metformin administration could be attributed to the highly expressed OCT3 in salivary glands [38].

4.3. Metformin clearance and elimination

Metformin is subjected to several organic cation transporters for its renal excretion in unchanged form such as (OCT1 and OCT2) [28, 38]. Metformin is also a substrate to the multidrug and toxin extrusion transporters (MATE-1 and MATE-2K) which play an important role in metformin secretory clearance into urine [30, 38]. Metformin renal clearance was dramatically reduced and a remarkable increase in its plasma concentration were noticed upon administration of pyrimethamine (MATE inhibitor) [30, 38].

In addition to their significant role in the metformin pharmacokinetics, metformin transporters (MATEs and OCTs) play an important role in predicting the clinical outcome of metformin co administered with other drugs due to possible drug-drug interaction and the overall pharmacological effect of metformin [30]. Diminished metformin uptake was noticed *in vitro* by the proton pump inhibitors through inhibition of OCT1, OCT2 and OCT3 [30]. *In vitro* inhibition of OCT1 was reported by two common antidiabetics (rosiglitazone and repaglinide) [30]. Renal clearance of metformin is decreased with cimetidine due to competitive excretion by OCT2 and possibly MATEs [28, 30].

5. Metformin pharmacodynamics

5.1. Role of metformin in the glycemic control

Liver seems to be a pivotal target organ for metformin's pharmacological actions [47]. The proclaimed effect of metformin in type II diabetic patients is the reduced hepatic glucose output mediated predominantly by inhibition of the gluconeogenesis [47]. The increased intestinal glucose utilization (non-oxidative) in addition to glucose disposal in skeletal muscles and adipose tissue are also crucial pharmacodynamic effects of metformin [47]. Alteration of the intestinal microbiota seem to be recent, yet important effect of metformin's actions in the gut [46-48].

Following its absorption, metformin reaches the portal vein at high concentration (40-70 µmol/L) which suggest the accumulation of high level of the biguanide in liver [46, 47]. Despite the slow hepatocyte uptake of metformin, its concentration within the liver cells and the mitochondrial matrix is 3-5 fold its concentration in the portal vein [46, 47]. High accumulation of metformin within mitochondria could be attributed to the cationic nature of the biguanide at physiological pH which facilitates its interaction with the

polarized membrane of the mitochondria [47]. The distribution of metformin in the cytosol of hepatocytes was also reported in literature which suggest multiple cellular mechanisms of metformin proposed for its effects [47].

Inhibition of mitochondrial oxidative phosphorylation is first hypothesized in 1960s for the biguanide cellular mechanisms [49, 50]. The results of 2 independent research groups in the 2000s emphasized that metformin reversibly inhibits complex 1 of the mitochondrial respiratory chain which resulted in decreased ATP levels and increased (AMP:ATP ratio) in the liver cells [51, 52]. These findings were supported by more recent studies on various cancer cell lines and human primary hepatocytes, where remarkable decline in mitochondrial NADH oxidation and decreased oxygen consumption rate were reported [47, 53-55]. Without triggering mitochondrial damage, metformin selectively induced a state in complex 1 to suppress energy transduction [56].

Cellular energy homeostasis is chiefly regulated by adenosine monophosphate activated protein kinase (AMPK), the cellular fuel gauge [45, 47]. The energy stress condition created by increased (AMP:ATP ratio) is a potential activator of AMPK [47]. Metformin induced AMPK activation in rat hepatocytes was first revealed in a key study in 2001 [57]. These findings were emphasized when the hypoglycemic effect of metformin was suppressed in absence of LKB1, the upstream kinase of AMPK in mouse model, which suggested that the gluconeogenic gene expression is inhibited by LKB1/AMPK signaling as a possible cellular mechanism of metformin [58]. Metformin induced chronic AMPK activation seem to play an important role in increasing hepatic and skeletal muscle sensitivity to insulin and inhibition of lipogenesis [47, 59, 60].

The hypoglycemic effects of metformin in hepatic AMPK deficient mice suggest that apart from the inhibitory effect on the gluconeogenic transcription, another mechanism (AMPK-independent) should be proposed for the decreased hepatic glucose output associated with metformin [61]. Cellular energy modulation and inhibition of the enzymes involved in the gluconeogenesis process are seemingly the acute effects of metformin in suppression of the hepatic glucose production process. The high energy required in the gluconeogenesis process could be dramatically affected by any decrease in the intracellular ATP levels, where six ATP equivalents are needed to synthesize one glucose molecule [47, 61]. The decreased hepatic production of glucose could also be attributed to another effect of metformin on the cytosolic and mitochondrial (NADH: NAD^+ ratio) [47]. Accumulation of AMP can potentially decrease glucagon-stimulated hepatic glucose output by inhibition of adenylate cyclase which resulted in decreased activity of protein kinase A (PKA) and its downstream signals [62]. Direct inhibition of mitochondrial glycerol-3-phosphate dehydrogenase seems to be another AMPK-independent mechanism that was proposed for metformin's inhibitory effect on the hepatic gluconeogenesis [63, 64]. **Figure 1** summarizes some of molecular mechanisms of metformin in glycemic control.

5.2. Role of metformin in cardiovascular protection

Compared to normoglycemic objects, diabetic patients are more prone to cardiovascular diseases, especially coronary heart disease [65]. Diabetic patients are at doubled risk of developing congestive heart failure (CHF) as they age [66]. The progression from asymptomatic left ventricular dysfunction to symptomatic CHF and the increased mortality risk was chiefly attributed to the hyperglycemic condition in diabetic patients [65]. Diabetes was reported by the National Cholesterol Education Program as CHF risk equivalent [67]. Death in diabetic patients is mainly attributed to the associated cardiovascular complications of the disease [68], where around 70% of diabetic patients die of cerebral and cardiac related macrovascular events [69].

Apart from its hypoglycemic effect, metformin seems to mediate certain cardiovascular protective actions through independent mechanisms [70]. Metformin was reported to decrease diabetes related death and overall mortality by 42% and 36% respectively [71]. Metformin was proven to protect against CHF during ischemia by multiple actions such as improved adaptation of cardiomyocytes metabolism, reduced cardiomyocytes apoptosis and enhanced myocardial preconditioning [72]. Metformin use was also associated with reduced infarct size and improved cardiac function in myocardial infarction animal model [73]. In a murine model of CHF, left ventricular function was significantly improved with metformin treatment [74].

Metformin was also suggested to lower the incidence of atherosclerosis and plaque formation in animals [30]. A significant decline in both diastolic and systolic blood pressures was noticed upon trialing metformin on non-diabetic, non-obese hypertensive objects. Interestingly, the metformin-mediated hypotensive effect lasted for two months of its discontinuation [75]. In a recent meta-analysis study on non-diabetic patients, metformin similarly lowered the systolic blood pressure of the tested group [76]. It was also reported that a reduction of 12% in diabetic complications could be seen with each 10 mm Hg decline in systolic blood pressure [77]. improved insulin sensitivity, reduced insulin levels, increased glomerular filtration rate and sodium secretion, adrenergic receptor blocking, reduced sympathetic activation or improved endothelial function are all proposed mechanisms for metformin-mediated decrease in the blood pressure [69].

T2D patients are manifested with generalized and impaired endothelial function due to decreased bioavailability of nitric oxide required for the regulation of vasomotor tone [3, 65]. The hyperglycemia-induced accumulation of ROS is claimed to be the primary trigger for impaired endothelial function and the subsequent vascular disease in T2D rather than the hyperglycemic condition itself [78]. Endothelial dysfunction characterized by reduced endothelium-dependent vasodilation, could be biochemically

identified through increased levels of certain biomarkers such as soluble vascular adhesion molecule-1 (sVCAM-1) and soluble intercellular adhesion molecule-1 (sICAM-1), where sICAM-1 and sVCAM-1 are key biomarkers in cardiovascular events [45]. Metformin was argued to improve the endothelial function by endothelial dependent blood flow and endothelial independent blood flow mechanisms [79, 80]. Metformin was also reported to decrease the levels of sICAM-1 and sVCAM-1 through mechanisms that are independent to its glycemic effects [78, 81] .

Type 2 diabetes is also considered as a procoagulant condition of disturbed hemostasis, increased coagulation and limited fibrinolysis [3]. T2D patients usually have increased blood viscosity and elevated levels of several coagulation factors such as fibrinogen, von Willebrand factor (vWF), increased platelet activation and aggregation in addition to increased level of plasminogen activator inhibitor-1 (PAI-1) [3]. Metformin therapy for T2D was reported to restore normal hemostasis by decreasing PAI-1, vWF, tissue type plasminogen activator, factor VII, factor XIII with direct effect on fibrin function and structure [65]. Metformin was also reported to decrease the circulating levels of PAI-1 and vWF of impaired endothelium of non-diabetic objects [82].

Metformin administration on the other hand, is associated with improved lipoprotein metabolism [65]. Decreased cholesterol, decreased LDL, and reduction of fasting and postprandial triglycerides are key features for metformin-mediated effects on lipid profile [30] [65]. Metformin is weight neutral, however weight loss can often be noticed with its use especially in subjects with impaired glucose tolerance [65].Weight loss associated with metformin administration could be attributed to improved insulin sensitization , reduced absorption of carbohydrates from GIT, glucagon-like peptide-1 anorectic effect or reduced levels of leptin and ghrelin [83, 84]. Metformin was also reported to redistribute fat, potentiate peripheral fat loss and reduce visceral abdominal fat accumulation in HIV associated lipodystrophy syndrome [85-87].

5.3. Role of metformin in polycystic ovary syndrome (PCOS)

PCOS is a metabolic and endocrinological disorder that affects 4-12% of females at the productive age [30] [69]. It is considered as one of the major causes of infertility in women who experience anovulation due to altered luteinizing hormone/follicular stimulating hormone ratio, hyperandrogenism and oligomenorrhoea [30, 31] [69]. A majority of PCOS women suffer from impaired glucose tolerance, high serum insulin levels and insulin resistance which puts them at high risk of developing T2D, metabolic syndrome and cardiovascular complications [31, 69].

Metformin was reported to reverse the metabolic abnormalities associated with PCOS where, restored ovulatory function, regular menstrual cycles, reduced circulating levels of androgen and insulin are all associated with the biguanide use [30] [69]. Improved ovarian estrogen secretion and facilitating sex hormone binding to globulin were two proposed effects of metformin in PCOS management [69]. Metformin was also reported to minimize the gestational diabetes and the frequency of miscarriage in PCOS candidates [30, 31]. Metformin was also reported to be included in some protocols of *in vitro* fertilization to improve ovarian stimulation and pregnancy chances [30].

5.4. Role of metformin in inflammation

Decreased serum levels of C-reactive protein (CRP) was observed in diabetic patients on metformin [30]. Metformin was proposed to interfere with the proinflammatory responses where it blocks the PI3K-Akt pathway by direct inhibition of NF-kB in vascular cell walls [65, 88]. Reduced production of tissue necrosis factor (TNF) and tissue factor (TF) were reported with metformin's use in some human monocytes exposed to oxidized low density lipoprotein and lipopolysaccharide [89]. Metformin administration to endotoxin-induced rat uveitis was associated with reduced production of inflammatory cytokines where monocyte chemoattractant protein-1α, TNF-α, macrophage inflammatory protein-1α and interleukin-1β were all decreased [90]. The decreased production of interleukin-6, TNF-α and interferon-γ from lipopolysaccharide-stimulated macrophages as a response to metformin was reported to be in a dose-dependent behavior [91].

Metformin use in rheumatoid arthritis management was also proposed where metformin inhibition of the mammalian target of rapamycin (mTOR) pathway was reported and reduced levels of TNF-α, interleukin-1 and inhibition of B and C cell proliferation were observed [30]. In plasma and monocytes of obese recipients of metformin, a noticeable decrease in the proinflammatory cytokine (macrophage inhibition factor) was reported [30]. The AMPK mediated anti-inflammatory effect of metformin was investigated by Reusse *et al*. who found a nociceptive analgesia from metformin comparable to that of ibuprofen [92].

5.5. Role of metformin in oxidative stress

Metformin was proposed to exert an antioxidant effect however the exact mechanisms are not clear [65]. Metformin-mediated inhibitory effects on complex 1 of the mitochondria seem to play an important role in the inhibition of the reactive oxygen species due to the inhibited mitochondrial respiration process [93-95]. In addition to the increased lipolysis and β-oxidation in adipocytes with metformin use, overexpression of uncoupled protein 2 (UCP2) and the increased level of reduced glutathione seem to play a pivotal role in reducing the oxidative stress associated with some conditions such as diabetes, inflammation and neurodegenerative diseases [93, 96].

5.6. Role of metformin in neuroprotection

Reduced levels of antioxidant enzymes and the high oxidative metabolism of human brain explains its vulnerability to oxidative environment [97]. Neurodegenerative ailments such as Alzheimer's disease characterized by accumulation of β-amyloid, are often described as type 3 diabetes (brain specific diabetes form) [65, 98] due to neuronal insulin resistance and impaired insulin homeostasis within the brain [65]. It was reported that AMPK-mediated action of metformin plays a significant role in neuroprotection against neurodegenerative diseases through induction of autophagy, neurogenesis and angiogenesis [99-102].

Low risk of cognitive impairment in T2D patients was reported with long term metformin therapy [103] with lower risk of dementia compared to other antidiabetic agents [104]. Metformin showed significant protection of working memory and domain of verbal learning in one study [105] and had antidepressant effect and improved the cognitive function in another study [106]. In 2019, American Diabetes Association (ADA) revealed that long-term metformin therapy in diabetic population protects against neurodegenerative damage [107].

5.7. Role of metformin in aging

It was reported that interfering with nutrient sensing pathways of mTOR and insulin/insulin-like growth factor (IGF-1) signaling, extended the lifespan of invertebrates [108]. Metformin was reported to retard aging and extend lifespan of *Caenorhabditis elegans* by regulation of systemic metabolism of microbial folate and methionine [46, 108]. Multiple mechanisms for metformin's action to retard aging were proposed [108], such as activation of AMPK, inhibition of mitochondrial complex 1,

reduced reactive oxygen species, inhibition of mTOR, reduced DNA damage, decreased insulin levels and interrupted IGF-1 signaling [30] [45, 69] [108]. Metformin was also proposed to exhibit its antiaging effect through interfering with cellular mechanisms and other metabolic pathways such as autophagy and alleviation of inflammation [109, 110]. In a mouse model, the mean life span of metformin treated mice increased by 8% and the maximum life span by one month compared to control group [111].

5.8. Role of metformin in cancer

T2D patients are at increased risk to cancer in multiple organs such as colon, pancreas, liver, breast, endometrium and bladder [112, 113]. Insulin resistance and elevated levels of insulin and IGF-1 (potential mitogenic) are the main tumorigenic factors in this population [114-117]. Metformin administration is associated with reduced levels of both insulin and IGF-1, so cell growth is limited in addition to metformin's ability to reduce other risk factors (insulin resistance and obesity) [30] [69].

T2D patients on metformin are at lower risk to develop cancer compared to non-metformin recipients [65]. Long-term administration of metformin to T2D women was associated with reduced breast cancer risk [118]. Diabetic patients who developed colorectal cancer exhibited lower mortality rate with metformin compared non-metformin patients [119]. The onset of carcinoma was delayed with an extended lifespan (mean of 8%) with metformin use in breast cancer model in mice [108]. The overall cancer incidence and cancer mortality were reported to drop by 31% and 34% respectively with metformin therapy [108].

Antineoplastic effect of metformin is predominantly thought to be mediated through LKB1/AMPK pathway [65], nevertheless inhibition of cell growth, inhibition of mTOR, inhibition of IGF-1, inhibition of angiogenesis, reduction of human epidermal growth factor receptor 2 (HER-2), induction of apoptosis, cell cycle arrest and inhibition of inflammation and angiogenesis are all proposed mechanisms to metformin-associated anticancer properties [65].

6. Biguanide-associated lactic acidosis

It is a clinical condition diagnosed when human plasma lactate exceeds 5 mmol/L and the blood pH drops below 7.35 [120]. Lactic acidosis was the main drawback of old

biguanides (buformin and phenformin) which was the main reason for their discontinuation [17]. Lactic acidosis is rare with metformin administration [69], but it could be fatal [28]. Renal function is a critical determinant in lactic acidosis incidence and metformin use is usually labelled with this warning [28]. Metformin administration in subjects with chronic renal impairment was recently updated by FDA who recommended monitoring glomerular filtration rate (eGFR) rather than creatinine clearance in metformin use [30] [121], where below eGFR of 30 ml/ min/1.73m^2, metformin should be contraindicated [30] [121].

7. The state-of-the art of microparticulate system in metformin delivery

Due to the growing interest in utilizing metformin beyond its glycemic effects in other clinical purposes such as cancer, aging and neurodegenerative diseases, there is a growing demand to optimize the oral metformin route to overcome the limitations of its oral pharmacokinetics (short half-life, incomplete and slow absorption, limited bioavailability and rapid elimination). Sustained release formulations were initially introduced to overcome the short half -life of metformin [28]. Although they seem to be more tolerated with less gastric side effects than immediate release metformin formulations, the rationale for such development is still not clear[14, 28]. Sustained or extended release metformin tablets are mainly retained by pylorus upon swelling of their matrix, so they only prolonged the gastric residence time of high metformin doses without significant improvement in the drug bioavailability [28] [122]. Osmotic sustained release tablets were also approached but, after single doses, low bioavailability was reported and similar to those immediate release formulations [28].

Small intestine was reported to be a significant site of metformin's action that could potentially be targeted, where metformin concentration measured within the small intestine was much higher than the concentrations in plasma and other tissues [28] [123]. Metformin's peak concentration in the jejunum was reported to be around 500 µg/ g of the tissue [124]. It was recently hypothesized that intestinal targeting over systemic circulation is responsible for most of the antihyperglycemic effects of metformin [46] [48] [125]. Furthermore, metformin-induced alterations of the gut microbiota seem to play a significant role in the therapeutic effects of the biguanide [48].

Buse *et al*. developed a formulation to delay the release of metformin till the distal small intestine at pH 6.5 or more using a proprietary coat [46, 125]. This delayed release formula was able to increase the hypoglycemic effect of metformin by 40% in addition to reducing the fasting plasma glucose level over 12 weeks [46]. Improved glycemia of T2D subjects over 16 weeks with delayed release metformin (targeting lower bowel)

was also reported in a similar study [126]. These findings suggest that intestinal targeting of metformin would necessitate newer dosage forms to be approached.

Polymeric particulate drug delivery systems such as microcapsules are innovative pharmaceutical dosage forms that have been recently implemented in the targeted delivery of their payloads to specific organ/tissue [127-137]. The free-flowing properties of microcapsules allow for preprogramed gastrointestinal transit time and reduced dose-dumping risk [127-137]. Of importance microcapsules offer valuable advantages in controlling, delaying, sustaining or prolonging the drug release kinetics of the encapsulated active pharmaceuticals with significant improvement in drug bioavailability [127-137]. On the other hand, they offer more protection against gastric irritation, improve the drug stability and mask unpleasant drug taste. Microcapsules could be defined as micron-sized (<1000 μm) particulates in which the drug core is enveloped in a polymeric coat or shell [127-137]. Several polymeric materials were reported in literature for drug encapsulation that can be of natural or synthetic origins [127-137]. **Figure 2** summarizes some of these polymers for potential applications in various encapsulation technologies.

Microencapsulation can be achieved chemically, physico-chemically or physico-mechanically by several methods such as coacervation and phase separation, pan coating, solvent evaporation, *in situ* polymerization, ionic gelation, spray drying and jet-flow ionic gelation [33, 127-138]. Various kinetic release mechanisms from microcapsules are proposed such as erosion, dissolution, degradation and diffusion [127-137]. In fact, these release characteristics depend not only on the drug nature, polymer nature but also on the microencapsulation process involved [127-137].

Generally, most active pharmaceutical agents can be formulated into microcapsules, however in order to optimize the therapeutic outcome, ideal drug candidates should possess short half-life and rapid elimination where these candidates are frequently administered for chronic symptoms [128, 132]. Metformin seems to be a good candidate for microencapsulation due to its inherent pharmacokinetic properties and the estimated release profile that can be designed, and site targeted with this technology to maximize its oral bioavailability and the expected therapeutic effects. **Table 2** summarizes some microencapsulation approaches for metformin.

8. The status quo of metformin microcapsules

Natural polymers such as alginate and pectinate were extensively used in the production of microparticulate drug delivery systems by several techniques [139-141]. Of

importance, orifice ionotropic gelation technique is considered one of the most utilized techniques in microcapsule fabrication [139-141]. The technique is generally based on the ionic reaction of negatively charged carboxylate terminals of α-L-glucuronic acid of these polymers with crosslinking solutions of multivalent cations to form egg box cross-linked structure [139-141]. The effect of alginate to metformin ratio on the release behavior and the physical properties of metformin loaded calcium alginate microspheres produced by ionotropic aerosolization technique was reported [142]. Low drug entrapment (<14%) and low drug loading (3.08%-3.99%) were noticed with the use of 0.5-1% (w/v) sodium alginate [142]. Increasing alginate to metformin ratio from (1:1) to (2:1), slightly improved metformin loading, entrapment efficiency, production yield and prolonged metformin release [142].

In another study, at a fixed alginate concentration (2% w/v), the total effect of the incorporated metformin amount was studied [143]. The authors reported that increasing metformin amount from (0.5 to 2% w/v) was associated with increase in microsphere size from (1.82 µm to 2.97 µm), improvement in metformin loading (from 5% to 15%) and decrease in both yield percentage from (80% to 59%) and encapsulation efficiency from (48% to 30%) [143].

Similarly, a polymer mixture of alginate/chitosan were prepared by solvent extrusion method and crosslinked with calcium chloride and citric acid to study metformin to polymer ratio in the produced microcapsules [144]. 2% w/v sodium alginate and 3% chitosan were used in the two formulations (M1 of 300 mg and M2 of 150 mg metformin). Narrow particle size distribution was recorded (600-800µm) [144]. Metformin content was higher in M1 with higher drug release over 12 hours [144]. Metformin release from both microcapsules was reported to follow first order kinetics for the first 6 hours followed by zero order kinetics thereafter [144].

In one step spray-drying process, metformin loaded microcapsules were prepared [145]. 2% sodium alginate solution loaded with metformin in a ratio of (1:2, polymer to drug) was cross linked by pouring into dilute calcium chloride solution to form metformin loaded calcium alginate dispersion [145]. The cross-linked alginate dispersion was then spray-dried to form metformin-loaded microparticles using Buchi mini spray dryer [145]. The produced microcapsules showed high entrapment efficiency (> 91%), high drug loading (> 75%) and metformin release was sustained for 12 hours in vitro and followed first order release kinetics [145].

The effect of different concentrations of calcium chloride and barium chloride in cross linking psyllium/sodium alginate polymeric skeleton by ionic gelation were studied, as well as the effect of other variables such as curing time (contact time between polymer and cross linker) on the particle size, swelling behavior and entrapment efficiency of the produced metformin loaded microcapsules [146]. Barium chloride crosslinked particles

were more spherical with regular surface, smaller in size and showed more prolonged metformin release in simulated intestinal fluid compared to calcium cross linked microcapsules [146].

Increasing the concentration of either calcium chloride or barium chloride produced tighter crosslinking characterized by smaller particle size, decreased matrix permeability, limited drug diffusion, less swelling in acidic conditions, and improved encapsulation efficiency especially when alginate content increased too [146]. Higher entrapment efficiency was recorded with a shorter curing time. Yet, elongation of curing time produced much smaller particles [146]. Barium crosslinked microcapsules prolonged metformin release in both gastric simulated and intestinal-simulated condition for up to 12 hours with less erosion than calcium crosslinked particles [146].

In a separate study, the same group investigated the release kinetics of psyllium/alginate metformin-loaded microcapsules and metformin microcapsules fabricated with sagu starch/alginate and cross linked with calcium chloride [147]. Microcapsules exhibited entrapment efficiency ranged from 40 to 62% with rough surface under scanning electron microscopy [147]. They also reported that the release of metformin from these microcapsules is chiefly dependent on metformin's diffusion through the polymeric skeleton of psyllium/alginate and sagu starch/alginate (non-Fickian diffusion drug release) [147]. Metformin release from the matrix of these microcapsules in phosphate buffer (pH 7.4) was prolonged to 12 hours [147].

In an attempt to control the release of metformin, the polymeric dispersion of *phoenix dactylifera* mucilage and sodium alginate was cross linked with zinc chloride by ionotropic gelation [148]. The microspheres produced were in the size range (0.44mm-1.99mm) [148]. The high viscosity of the polymeric dispersion improved the entrapment efficiency and more swelling and larger particle size were noticed with increased alginate to mucilage ratio [148]. Metformin entrapment efficiency was in the range (25-91%) [148]. The formulations with alginate to mucilage ratio of (2:1) and (3:1) were able to control metformin release for 24 hours in a non-Fickian diffusion mechanism [148].

Gastro-retentive microparticulate drug delivery systems is another approach to control, prolong or sustain metformin release and improve its pharmacokinetic properties [149]. The approach aims to increase the residence time of metformin microcapsules by floating over the gastric fluid, adhesion to the mucosal wall or both [149, 150].

A group of researchers developed gastro-retentive buoyant metformin-loaded microcapsules through the incorporation of calcium carbonate (a gas forming agent) in 2% w/v sodium alginate that when reacted via ionic gelation with acidified calcium chloride solution, carbon dioxide evolved and trapped within the polymer matrix [151].

The yield production was more than 85% and encapsulation efficiency was quite high (>77%) [151]. All microcapsules produced with different alginate to calcium carbonate ratio were porous, spherical in shape with particle size ranged from 861µm-991µm and immediately floated in simulated gastric fluid for up to 8 hours [151]. Formulation with alginate to carbonate ratio of (1:1) showed highest buoyancy percentage, sustained metformin release in simulated gastric fluid for 8 hours and prolonged the hypoglycemic effect in diabetic rats compared to non-floating microcapsules prepared without calcium carbonate [151].

In a similar study, Salunke *et al*. compared the characteristics of ionotropically gelled metformin microcapsules prepared with 2 different gas-generating agents (calcium carbonate and sodium carbonate) when ethyl cellulose and hydroxypropyl methyl cellulose (HPMC) K4M were separately mixed with sodium alginate [152]. They reported that calcium carbonate showed a better ability to generate carbon dioxide in the *in vitro* acidic conditions with better buoyancy performance [152]. They also reported that HPMC K4M showed superior metformin entrapment over ethyl cellulose in the prepared microcapsules [152]. Microcapsule size was in the range 447.1- 801.8µm, drug loading and entrapment efficiency exceeded 34% and 37% respectively for all tried formulations. The formulation prepared with alginate, HPMC, ethyl cellulose and calcium carbonate at a total polymer to calcium carbonate ratio of (1:1 w/w) floated for up to 24 hours and sustained metformin release over this period [152].

Floating microcapsules of metformin were also approached by Pandit *et al*. by emulsification solvent evaporation technique using ethyl cellulose [153]. The optimized formulation prepared with metformin to ethyl cellulose ratio of (1:2) showed the highest entrapment efficiency (82.28%), the best floating performance (>99% floated up to 5 hours), the highest drug content (>95%) and sustained metformin release over 12 hours [153]. The gastric residence time for the optimized formulation was reported to be more than 22 hours. Upon oral administration of this formulation to diabetic Wistar rats, bioavailability was improved and a significant decline in blood glucose level was noticed over one month [153].

Choudhury *et al*. developed metformin loaded floating cellulose acetate microspheres by emulsification solvent evaporation to improve metformin bioavailability and to extend its release [154]. The production yield ranged 45-70% and microspheres remained buoyant for over 10 hours [154]. High entrapment efficiency (>75%) was reported for different formulations trialed [154]. Formulations prepared with metformin to ethyl cellulose ratio of (1:3 and 1:5) exhibited 90% metformin entrapment efficiency and better buoyancy profile [154]. *In vivo* results following oral administration of these microcapsules to male albino mice was associated with a significant hypoglycemia over 10 hours compared to pure metformin [154].

The intimate contact of a microparticulate system with gastric and/or intestinal mucosa is an acceptable approach to increase the drug residence time and drug uptake at a specified absorption site [117]. This approach can be achieved by several bio-adhesive polymeric materials to improve the pharmacokinetic properties of antidiabetic agents [117] [155].

In one approach, sodium alginate was blended with different mucoadhesive polymers (Carbopol 934p, hydroxy propyl methyl cellulose and carboxymethyl cellulose sodium) at different ratios to encapsulate metformin by ionic gelation process [156]. The produced microcapsules had entrapment efficiency range of (65-80%) with good mucoadhesive properties in an *in vitro* wash off test from rat intestinal mucosa [156]. Metformin release was prolonged over 16 hours [156].

Similarly, another group of researchers encapsulated metformin by ionotropic gelation reaction of sodium alginate with chitosan, ethyl cellulose, HPMC and Carbopol 934p [157]. HPMC formulated microcapsules had the highest bioadhesion in simulated gastric fluids while Carbopol microcapsules exhibited better bioadhesion in simulated intestinal fluid [157]. The formulation prepared with alginate: Carbopol at the ratio of 1:3 showed the most sustained *in vitro* release effect of metformin over 15 hours [157]. Similar results were reported by researchers who utilized ionotropic gelation to encapsulate metformin with the same set of polymers but replaced ethyl cellulose with gelatin [158]. They reported that the formulations made with alginate to Carbopol at ratios 9:1 and 9:2 showed the best mucoadhesive characteristics and were able to prolong metformin release over 10 hours [158].

Gum karaya and alginate were combined to formulate mucoadhesive metformin microcapsules by two different techniques, ionotropic gelation and emulsification gelation process [159]. Microcapsules produced with emulsification gelation process were superior in sustaining metformin release in 0.1 N HCl over 12 hours [159]. Both formulations showed good mucoadhesive properties in an *in vitro* wash off test from sheep stomach mucosa over 8 hours [159]. The release behavior from all gum karaya formulations followed zero order kinetics with non-Fickian transport [159].

Nayak *et al*. developed metformin-loaded mucoadhesive microcapsules made of alginate and tamarind seed polysaccharide [160, 161]. The authors used 3^2 factorial design to optimize the ionotropically-gelled microcapsules [160, 161]. The optimized microcapsules showed more than 94% metformin entrapment efficiency with good mucoadhesive properties to biological membranes [160, 161]. These microcapsules showed pH-dependent swelling behavior, where swelling in phosphate buffer was much higher than swelling in HCl [161]. The optimized microcapsules followed zero-order kinetic release over 10 hours [160, 161] with significant hypoglycemia in alloxan-induced diabetic rats following oral administration [160, 161]. Same results were

reported by Nayak et al. upon conducting similar experiment with low methoxy pectin and tamarind seed polysaccharide [162]. The reported encapsulation efficiency was slightly higher than that in alginate microcapsules (> 95%) [162].

The mucoadhesive properties of fenugreek seed mucilage to encapsulate metformin were studied with gellan gum [163] and low methoxy pectin [164] by ionic gelation. The developed microcapsules were optimized by 3^2 factorial design [163, 164]. The optimized microcapsules showed pH-dependent swelling (higher swelling in simulated intestinal conditions), had good mucoadhesive properties to goat intestinal mucosa and sustained metformin release for up to 10 hours [163, 164].

Carbomer 934p and ethyl cellulose at ratio 3:1 were used to formulate mucoadhesive metformin microcapsules by emulsification solvent evaporation method [165]. The produced microcapsules prolonged metformin release for 8 hours [165]. In a similar approach, emulsification solvent evaporation technique was utilized to fabricate microcapsules with ethyl cellulose and Carbopol 934p at ratios (1:1) and (2:1) [166]. The authors reported good mucoadhesive properties of these microcapsules to goat intestinal mucosa and sustained metformin release over 12 hours [166].

Alginate, pectin, polyacrylic acid and poly (lactic-co-glycolic acid) were randomized by Box-Behnken statistical design to formulate dual system metformin microspheres (gastro-adhesive and gastro-floatable) by ionic gelation [150]. All microspheres remained buoyant for more than 8 hours in simulated gastric fluids and prolonged metformin release for more than 11 hours [150].

Ethyl cellulose was used to encapsulate metformin by emulsification solvent evaporation technique [167]. Microcapsules made with drug to polymer ratio of 1:5 showed the highest encapsulation efficiency (69%) and sustained metformin release in phosphate buffer for 8 hours [167]. In a similar work, the viscosity effect of ethyl cellulose used to encapsulate metformin by emulsion solvent evaporation was studied [168]. Microcapsules formulated with ethyl cellulose of viscosity (40-42cps) and at a ratio of 1:6 (drug to polymer) showed the highest encapsulation efficiency (>97%) and sustained metformin release for more than 12 hours [168]. Another group of researchers studied formulation variables influencing metformin-loaded microcapsules prepared with ethyl cellulose (18-22cps) by emulsification solvent evaporation technique [169]. Increasing the stirring speed from 400 to 800 reduced the particle size significantly (from 846 µm to 134 µm) and increasing the surfactant (span 80) from 2-6 % v/v reduced the particle size from 954 µm to 242 µm [169]. Metformin content ranged from 45-75% and the release from different microcapsules showed biphasic release (initial burst release of metformin followed by sustained pattern over 6 hours) [169].

Choudhury *et al*. produced ethyl cellulose coated metformin microspheres utilizing two different techniques, emulsion solvent evaporation and non-solvent addition [170]. Microcapsules prepared with non-solvent addition had higher production yield, higher metformin entrapment and sustained metformin release *in vitro* for over 10 hours [170]. On the other hand, microcapsules produced by emulsification solvent evaporation had smoother surface under scanning electron microscopy with better glycemic control *in vivo* in alloxan-induced diabetic albino mice [170].

A polymeric blend of ethyl cellulose and polyethylene glycol was investigated to encapsulate and prolong metformin release from different microcapsules [171]. The authors utilized emulsion solvent evaporation technique in the encapsulation process [171]. Microcapsules formulated at polymer mix to drug ratio of 6:1 and at ethyl cellulose to polyethylene glycol ratio of 4:1 showed the highest production yield (81.7%), highest metformin loading (82.1%) and the maximum entrapment efficiency (82.13%) [171]. All microcapsules exhibited initial burst release in the first 3 hours followed by prolonged release over 9 more hours that followed Higuchi kinetic model with Fickian diffusion [171].

Cao *et al*. developed sustained release metformin microcapsules by Wurster fluidized bed technique [172]. The authors used ethyl cellulose (45 cps or 100 cps) as a coating material with the addition of a plasticizer (glycerol triacetate or dibutyl sebacate) [172]. A cation exchange resin was homogenized with metformin solution which is then blown into a Wurster tube with a spray gun to produce free-flowing metformin microcapsules [172]. They reported that these microcapsules sustained metformin release over 10 hours [172].

Hassan *et al*. prepared two types of polymethacrylate/metformin microcapsules by emulsification solvent evaporation method [173]. The authors reported that the microcapsules made with Eudragit RLPO exhibited first order release kinetic with Fickian transport [173]. On the other hand, Eudragit RSPO formulated microcapsules showed better sphericity with smoother surface, entrapped more metformin (88-97%) and exhibited higher release retardation of metformin over 10 hours (anomalous release non -Fickian transport) [173].

In a study to compare the *in vivo* pharmacodynamics of different metformin-loaded microparticles, hydroxypropyl methyl cellulose K100, ethyl cellulose, compritol 888 ATO, Eudragit RL and Eudragit RS were used to coat metformin by solvent evaporation method [174]. The authors used 2^2 factorial design to optimize their formulations [174]. The microcapsules prepared with metformin and compritol 888 ATO at ratio 1:4 was the optimized formula that showed 97.6% metformin content, sustained metformin release up to 12 hours *in vitro* and showed significant hypoglycemia for 12 hours in an animal model [174].

A group of researchers formulated metformin-loaded microcapsules by complex coacervation technique and optimized the formulations with response surface methodology [175]. Metformin solution was emulsified with sunflower oil and eventually coated with pectin and soya bean protein isolate [175]. The optimized formula had a particle size of 16 μm and the process yield was up to 84% [175].

In a novel approach, Gedawy *et al*. developed silicon-based metformin microcapsules [33]. The authors developed self-emulsified polydimethylsiloxane grafted with alginate to encapsulate metformin with vibrational jet nozzle ionotropic gelation technology [33]. The process had high production yield (> 97%) and all formulations recorded higher metformin loading, higher encapsulation efficiency and better particle size distribution curve compared to control [33]. The siliconized microcapsules exhibited less swelling in simulated gastric fluid for up to 4 hours and reached maximum swelling in simulated intestinal fluid in one hour [33]. The new metformin-loaded silicone microcapsules had a better mechanical strength than control in phosphate buffer (pH 6.8) over 8 hours [33].

9. Conclusion

Since its inclusion in the essential medicine list of WHO in 2011, metformin has become the most recommended antidiabetic agent for type 2 diabetes. This biguanide, on the other hand, has gained special importance in non-diabetic conditions with promising future in new clinical applications of oncology, aging and Alzheimer's disease. The pharmacokinetic characteristics of metformin necessitate its frequent oral administration and sometimes at high doses which is normally accompanied with gastric side effects. Microparticulate delivery systems utilizing various polymers offer a promising versatile tool in optimizing the oral bioavailability of metformin, reduce the repeated administration by prolonging and or/targeting the drug release and protect against gastric symptoms. Further investigations in the polymeric particulate drug delivery systems are still required not only to develop new platforms to maximize the pharmacological benefits of metformin but also to target the biguanide to different tissues or organs.

10. Future perspective

The pharmacodynamics of metformin are quite interesting, where the pharmacological effects of this unique biguanide are not limited to its antihyperglycemic effects. Recently, a significant number of research papers and studies revealed the role of metformin in different pathologies including geroprotection, oncology, inflammation, polycystic ovary syndrome and neurodegenerative diseases. To date, the detailed molecular mechanisms of metformin in these conditions have not been revealed. Further

investigations and clinical trials are required to provide more information about non-diabetic guidelines in using metformin as well as doses required in different ailments. On the other hand, the research in pharmaceutical development and improvement of metformin's dosage forms are critical requirements in improving its oral bioavailability. Innovative approaches in polymeric micro/nanoparticulate platforms are promising tools that can be employed to overcome the pharmacokinetic limitations of metformin and facilitate organ/tissue targeting.

Executive summary

- Metformin belongs to Class III materials as per Biopharmaceutics Classification System (BCS) as well as Biopharmaceutics Drug Disposition Classification System (BDDCS).
- According to Salivary Excretion Classification System (SECS), metformin is considered as class II therapeutic.
- Due to its availability as hydrophilic cation at physiological pH, metformin is not expected to diffuse passively across cell membrane.
- Absorption, distribution, elimination and some pharmacological effects of metformin are controlled by various transporters.
- Metformin is predominantly absorbed from proximal small intestine, where negligible amount of this biguanide is absorbed from large intestine or stomach.
- Flip-flop pharmacokinetics are characteristic for metformin.
- Higher doses of metformin result in less bioavailability due to decreased rate of absorption.
- No evidence of metformin plasma protein binding, where high volume of distribution of this biguanide is noticed.
- Metformin is excreted unmetabolized in human urine.
- Inhibition of gluconeogenesis and reduction in the hepatic glucose output is the primary effect of metformin in glycemic control.
- Reversible inhibition of mitochondrial complex 1 is a paramount cellular effect of metformin.
- Chronic activation of AMPK by metformin could induce sensitization of skeletal muscles and hepatocytes to insulin.
- A significant cardiovascular protective effect of metformin has been evidenced apart from its hypoglycemic action.
- Metformin administration is associated with decreased production of different interleukins and other inflammatory mediators.
- Reduced oxidative stress is mediated by the antioxidant effect of metformin.

- Improved cognitive function was reported with metformin use.
- Several mechanisms were hypothesized for metformin anti-aging effect.
- Metformin has been trialed in multiple cancer models with promising results.
- Microparticulate drug delivery systems are promising dosage forms that can be utilized in improvement of the pharmacokinetic properties of different pharmaceuticals and allow for tissue/organ targeting.

Conflict of interest

None to declare.

Financial and competing interests

None to declare.

Figure and table legends

Figure 1. Some glucoregulatory mechanisms of metformin in hepatocytes. **AMPK**: AMP-activated protein kinase. **GLUT2**: Glucose transporter 2. **LKB1**: Liver kinase B1. **OCT1** and **OCT3**: Organic cation transporters. **PKA**: cAMP-dependent protein kinase.

Figure 2. Polymers and coating materials in microparticulate drug delivery system.

Table 1. Transporters involved in the pharmacokinetics of metformin.

Table 2. Recent microencapsulation approaches for metformin.

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** This paper is the first to document comprehensive multifacted stability profiling of metformin with sulfonylureas under various stress condtions and it can be used either in research protocols or in quality control and pharmaceutical industry of various dosage forms of metformin, glipizide and gliclazide.

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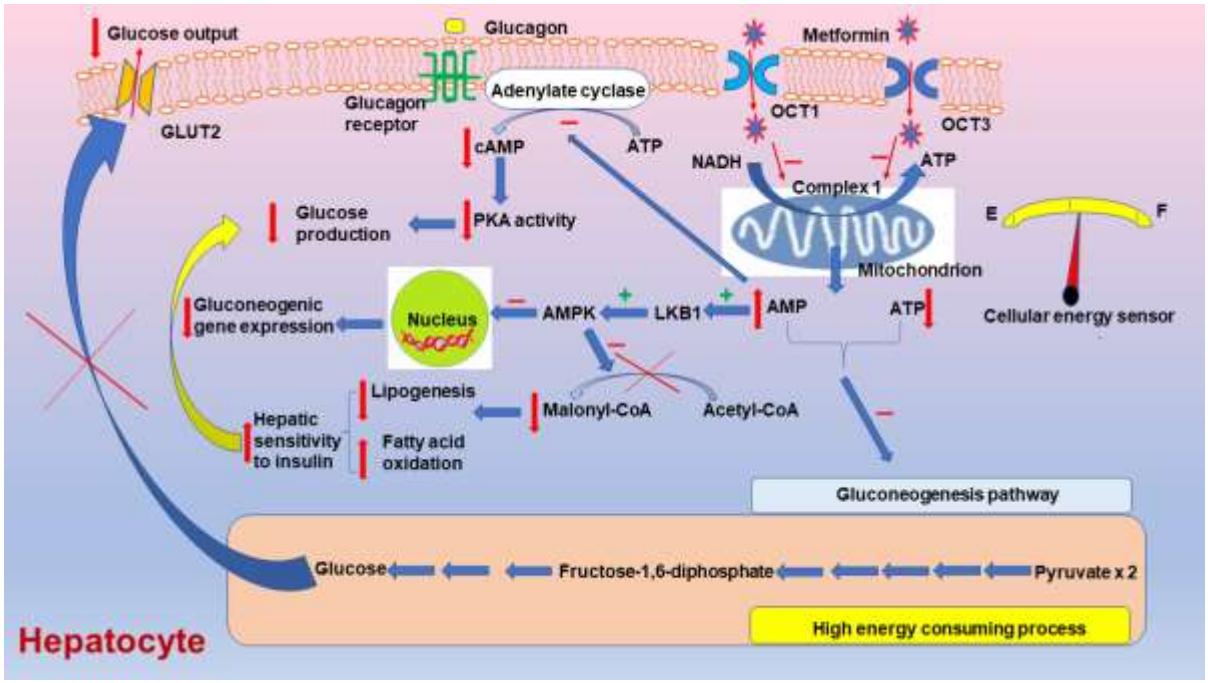

Figure 1. Some glucoregulatory mechanisms of metformin in hepatocytes. **AMPK**: AMP-activated protein kinase. **GLUT2**: Glucose transporter 2. **LKB1**: Liver kinase B1. **OCT1** and **OCT3**: Organic cation transporters. **PKA**: cAMP-dependent protein kinase.

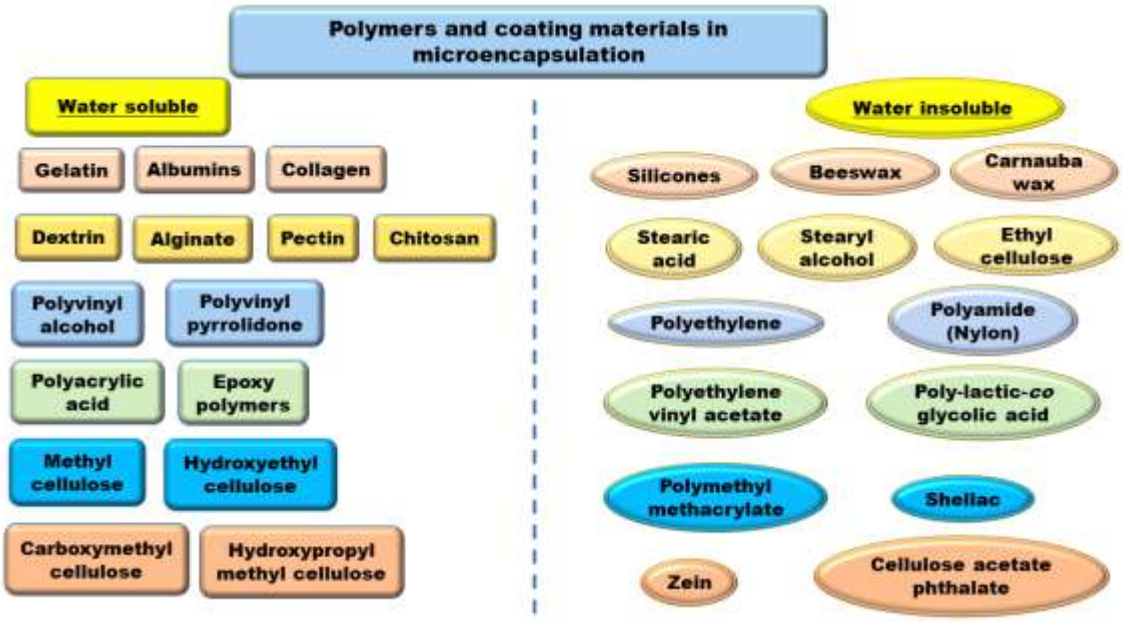

Figure 2. Polymers and coating materials in microparticulate drug delivery system.

Table 1. Transporters involved in the pharmacokinetics of metformin.

Transporter	Major tissue/organ	Role	Reference
OCT1	Basolateral membrane of enterocytes	Intestinal absorption of metformin from enterocytes into bloodstream	[28, 29, 30, 38]
	Sinusoidal membrane (basolateral side) of hepatocytes	Hepatic uptake of metformin	
	Luminal side of proximal and distal tubules	Renal excretion of metformin and/or resorption	
OCT2	Basolateral membrane (blood side) of renal tubule cells	Metformin entry into proximal tubular lining cells for subsequent urinary metformin excretion	
OCT3	Brush borders of enterocytes	Transport of metformin into enterocytes	
	Sinusoidal membrane (basolateral side) of hepatocytes	Hepatic uptake of metformin	
PMAT	Luminal side of enterocytes (Apical membrane)	Gastrointestinal uptake of metformin and its transport into enterocytes	
OCTN1			
SERT			
THTR-2			
MATE-1	Apical membrane (brush borders) of the renal proximal tubule cells	Metformin transport from tubular lining cells into urine	
MATE-2K			

Table 2. Recent microencapsulation approaches for metformin.

Polymers and materials used	Encapsulation technique	Major features	References
Sodium alginate (0.5- 1 % w/v)	Ionotropic aerosolization	Low drug loading (<4%). Low encapsulation (<14%)	[142]
Sodium alginate (2% w/v)		Increasing amount of metformin added in formulation improved drug loading (5-15%)	[143]
Sodium alginate and psyllium husk	Ionic gelation with $CaCl_2$ and $BaCl_2$	High metformin entrapment upon increasing alginate and cross-linker concentration ($CaCl_2$ or $BaCl_2$). Barium cross-linked alginate is superior in prolonging metformin release over calcium.	[146]
Sodium alginate, Sagu starch and psyllium husk	Ionic gelation	40-60% encapsulation efficiency.	[147]
Sodium alginate and chitosan	Solvent extrusion	Prolonged metformin release (12 hours)	[144]
Sodium alginate and date mucilage	Ionotropic gelation	25-91% entrapment efficiency. Controlled metformin release (24 hours)	[148]
Sodium alginate, hydroxy propyl methyl cellulose and $CaCO_3$	Ionotropic gelation (buoyant microspheres)	>77% metformin entrapment efficiency. Sustained metformin release (8 hours)	[151]
Sodium alginate, hydroxy propyl methyl cellulose, ethyl cellulose, $NaHCO_3$ and $CaCO_3$		$CaCO_3$ microcapsules had better floating properties. 34-48 % metformin loading.	[152]
Ethyl cellulose	Emulsion solvent evaporation (buoyant microspheres)	>94% metformin content. Sustained release over 12 hours	[153]
Cellulose acetate		73-98% encapsulation efficiency. Sustained metformin release > 10 hours.	[154]
Sodium alginate, carboxy methyl cellulose Na, Carbopol 934p and hydroxy propyl methyl cellulose	Ionic gelation (mucoadhesive microspheres)	65-80% encapsulation efficiency. Sustained hypoglycemic effect in vivo for 16 hours	[156]
Sodium alginate, ethyl cellulose, hydroxy propyl		Carbopol based formulation with highest bio-adhesion sustained metformin release over 15 hours.	[157]

methyl cellulose, chitosan and Carbopol 934p			
Sodium alginate and gum karaya	Ionotropic gelation and emulsion gelation (mucoadhesive microcapsules)	Release retardation was more pronounced in microcapsules produced by emulsion gelation process over 12 hours.	[159]
Sodium alginate and tamarind seed polysaccharide	Ionic gelation (mucoadhesive microspheres)	>94% metformin encapsulation. Sustained in vitro release for 10 hours	[160, 161]
Low methoxy pectin and fenugreek seed mucilage		Significant hypoglycemia in diabetic rats over 10 hours	[164]
Sodium alginate, pectin, polyacrylic acid and poly (lactic-co-glycolic acid)		Buoyant microcapsules for 8 hours that sustained metformin release for 11 hours	[150]
Sodium alginate	Spray drying/ionic gelation	>90% encapsulation efficiency. Sustained release for up to 12 hours	[145]
Ethyl cellulose and polyethylene glycol.	Emulsion solvent evaporation	>82 % metformin loading. Sustained drug release over 12 hours	[171]
Eudragit RSPO and Eudragit RLPO		77-97% encapsulation efficiency. Eudragit RSPO was superior in sustaining metformin release up to 10 hours.	[173]
Ethyl cellulose, glycerol triacetate and dibutyl sebacate	Wurster fluidized bed	Sustained metformin release for 10 hours	[172]
Pectin, soybean protein isolate	Complex coacervation	Production yield >80%	[175]
Sodium alginate and polydimethylsiloxane	Vibration jet nozzle ionotropic gelation	Production yield >97%. Higher metformin content and encapsulation efficiency compared to control	[33]

Chapter 2

Graphical abstract

Chapter 2: Polydimethysiloxane-customised nanoplatform for delivery of antidiabetic drugs

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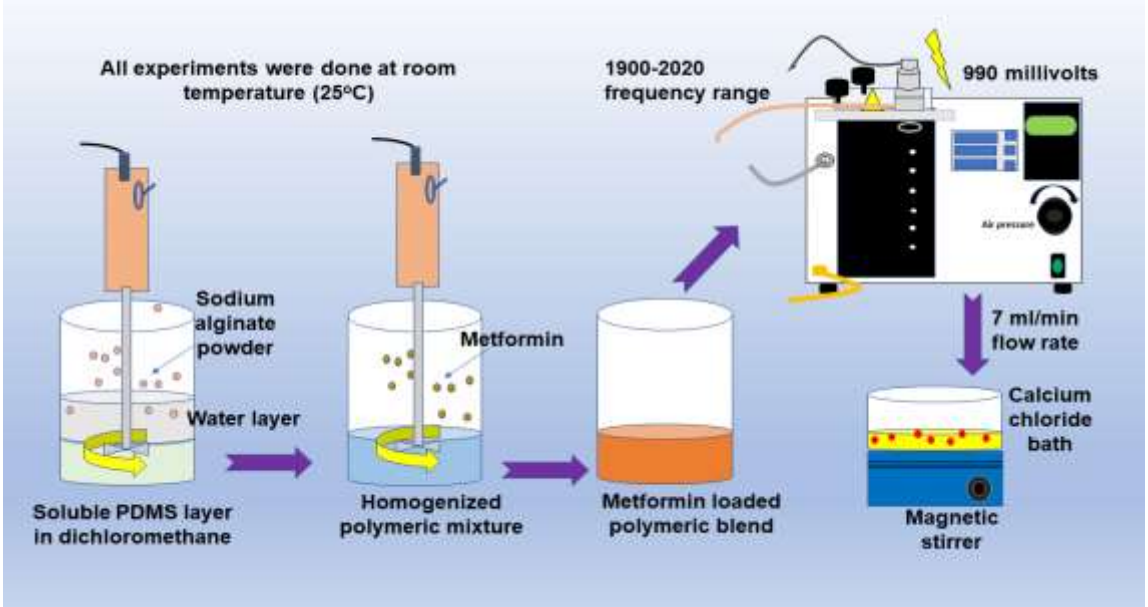

This chapter outlines the production process of polydimethylsiloxane customised metformin microcapsules by vibrational jet flow ionotropic gelation technology and characterisation of these microcapsules in terms of rheological properties, particle size distribution, optical microscopy, scanning electron microscopy, energy dispersive x-ray, swelling properties, mechanical resistance, yield production, metformin loading and encapsulation efficiency.

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Therapeutic Delivery

Polydimethylsiloxane-customized nanoplatform for delivery of antidiabetic drugs

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Aim: To develop a new self-emulsified silicon-grafted-alginate platform for pharmaceutical delivery. The produced biocompatible polymeric blend would be used to encapsulate metformin by a vibrational jet-flow ionotropic gelation process. **Materials & methods:** Polydimethylsiloxane was homogenized with alginate to prepare a stable polymeric mixture to which metformin was added. A metformin-loaded polymeric vehicle was then pumped through Buchi B-390 into $CaCl_2$ to produce microcapsules. **Results & conclusion:** The platform showed a powerful, pseudoplastic thixotropic and demonstrated strong, efficient and wide applications of polydimethylsiloxane-customized technology in drug delivery and stability. A substantial improvement in drug loading, encapsulation efficiency and flow properties were noticed in siliconized microcapsules compared with the control.

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Keywords: alginate • antidiabetics • delivery • encapsulation • ionic gelation • metformin • organosilicon • PDMS • platform

Metformin, in addition to its substantial role in Type 2 diabetes as the first-line therapy [1], has gained pharmacological interest in nondiabetic conditions such as polycystic ovary syndrome, geroprotection and oncology [1,2]. Metformin belongs to the basic biguanides family (pKa 12.4) and its hydrochloride salt has a molecular mass of 165.6 g/mol and is freely water-soluble (1.38 mg/ml) [3]. Metformin undergoes incomplete and slow absorption after oral administration with limited oral bioavailability (40–60%) [4–7]. Absorption of metformin from the stomach and large intestine is negligible, yet the small intestine seems to be the optimal site of its absorption [4–7]. Metformin administration is also associated with gastric irritation [8]. Microcapsules as a dosage form can withstand acidic conditions of the stomach and can be designed to deliver their payloads at a higher pH of the intestine or beyond [9–16], they are also believed to modify and target drug release [9–16]. Microcapsules are also reported to maintain drug stability, improve drug bioavailability, minimize gastric symptoms and improve patient compliance to prescribed therapeutics [9–16]. The literature reveals several polymers which have been utilized in the preparation of metformin-loaded microcapsules such as methoxy pectin, chitosan, Gum karaya, Carbopol 974p, sodium alginate, polyacrylic acid, hydroxy propyl methyl cellulose, poly(lactic-*co*-glycolic) acid, Eudragit S100 and Eudragit RL100 [17–22]. On the other hand, versatile platforms and different techniques were employed to entrap other antidiabetic agents [23,24].

Silicone polymers known as polysiloxanes or polydimethylsiloxanes (PDMS) have extensively been used in theranostics (diagnosis and therapeutic aid), drug delivery, cosmetology, aesthetics and pharmaceutical and biomedical applications due to their biocompatibility, versatility, physicochemical stability [25], resistance to UV radiation and high gas permeability [26]. The aforementioned characteristics of PDMS may be attributed to their unique hybrid structure (nonpolar organic functional groups attached to inorganic polar skeleton) [25,27]. Silicon polymers could occasionally be used as active pharmaceutical agents such as simethicone and dimethicone [27], in pharmaceutical excipients, in contact lenses or drug reservoir implants and intravaginal rings [27]. Due to their film-forming ability,

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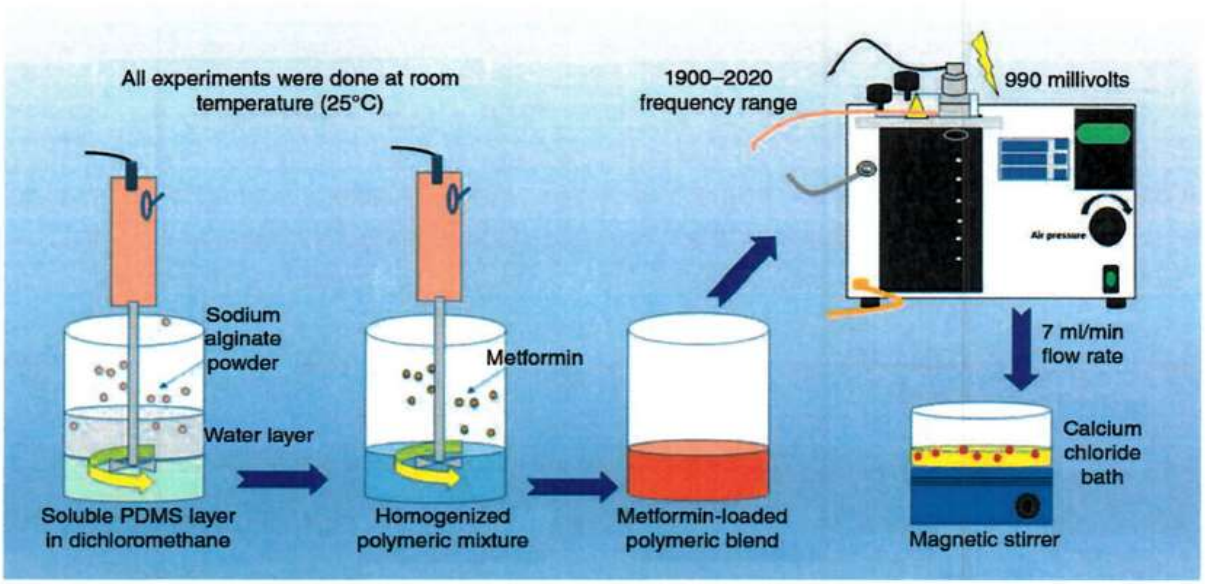

Figure 1. Emulsifying polydimethylsiloxane with alginate and production of metformin-loaded microcapsules through vibrating laminar jet-flow ionotropic gelation.
PDMS: Polydimethylsiloxane.

PDMS polymers have also been used in pharmaceutical coating [25,28–30]. Silicon polymers also gained importance in transdermal and topical drug delivery due to their wettability and hydrophobicity [31].

Sodium alginate is a biocompatible natural hydrophilic polysaccharide that is frequently used in pharmacy and in bioencapsulation [32,33]. In this study, we developed a novel platform comprising two polymers of opposite natures PDMS (hydrophobic) and sodium alginate (water soluble) into a stable self-emulsified polymeric vehicle to encapsulate metformin (the model drug). Microcapsules were produced using laminar jet-flow technology [34,35], with the self-emulsified silicon polymer based on a ionotropic gelation reaction of (-COO^-) terminals of α-L-guluronic acid (from alginate blocks of silicon/alginate matrix) cross-linked with Ca^{2+} of $CaCl_2$ to form a 3D network [32]. To the best of our knowledge, this is the first report of production of silicon-based microcapsules from PDMS-grafted alginate without the need of silicon copolymers to cross-link a silicon oil (PDMS). Drug-loading capacity and encapsulation efficiency of some polymeric vehicles could be problematic and there is an increasing demand in developing new therapeutic platforms.

Experimental

Materials

Metformin hydrochloride, PDMS (100 mPa.s) and $CaCl_2$ (anhydrous, 96% pure) were purchased from ThermoFisher Scientific (Melbourne, Australia), medium viscosity sodium alginate was procured from Sigma-Aldrich (MI, USA) and polycarbophil (PCP; Noveon® AA-1) was gifted from Noveon (OH, USA). All other solvents and reagents were of HPLC grade.

Preparation & formulation of metformin-loaded microcapsules

Preparation of metformin-loaded polymeric mixture

PDMS was solubilized in an equal amount of dichloromethane. Sodium alginate powder, PCP (only for PCP-containing formulations) and water was added to the organic PDMS layer and homogenized (Ultra-Turrax homogenizer, Germany) for four cycles (1 min each, 30 s apart) (Figure 1). Metformin HCl was then added to the final homogenized polymeric blend and homogenized for 1 more minute. The total polymeric mass to metformin HCl was maintained at 2:1 ratio for all formulations(Table 1). The control sample was prepared by addition of metformin HCl to a freshly homogenized aqueous solution of sodium alginate.

Table 1. Polymeric coat composition and ratios of sodium alginate, polydimethylsiloxane and polycarbophil of different formulations encoded as (control, A, B, C, K, L and M), their production yield, metformin loading, encapsulation efficiency, Carr's index and Hausner ratio of the produced microcapsules.

Formula code	Microcapsule coat composition for 100 ml water				Mean ± SD (n = 3)				
	Sodium alginate (mg)	PDMS (mg)	Polycarbophil (mg)	Metformin (mg)	Production yield (%)	Metformin loading (%)	Encapsulation efficiency (%)	Carr's index (%)	Hausner ratio
Control	1500	–	–	750	97.7 (±0.10)	3.46 (±0.05)	10.38 (±0.14)	12.93 (±0.52)	1.148 (±0.001)
A		1500	–	1500	98.2 (±0.06)	5.99 (±0.13)	17.99 (±0.39)	12.79 (±0.52)	1.147 (±0.007)
B		750	–	1125	97.8 (±0.10)	4.92 (±0.15)	14.77 (±0.44)	14.29 (±0.76)	1.167 (±0.010)
C		375	–	937.5	98.1 (±0.06)	4.23 (±0.08)	12.69 (±0.25)	13.51 (±0.63)	1.156 (±0.008)
K		1500	100	1550	98.5 (±0.15)	5.95 (±0.19)	17.85 (±0.59)	11.58 (±0.07)	1.131 (±0.001)
L		750	100	1175	99.3 (±0.15)	5.59 (±0.10)	16.76 (±0.30)	11.86 (±0.08)	1.134 (±0.001)
M		375	100	987.5	97.9 (±0.06)	4.93 (±0.13)	14.78 (±0.38)	12.09 (±0.08)	1.137 (±0.001)

PDMS: Polydimethylsiloxane; SD: Standard error.

Preparation of metformin-loaded microcapsules

Microcapsules loaded with metformin HCl were prepared by pumping the freshly prepared metformin-loaded polymeric blend at a flow rate of 7 ml/min (Buchi B-390 encapsulator, Switzerland), utilizing the laminar vibrating jet-flow, operated at a frequency range of 1900 to 2020 and 990 mV, into a gently agitated 5% $CaCl_2$ bath. Microcapsules were kept in $CaCl_2$ for 15 min to complete the curing reaction. They were then collected by decantation and washed twice with 150 ml Milli-Q water, then dried in a thermostat room at 37°C for 1 week (till complete dryness) and kept in a desiccator till they were used.

Characterization of drug-loaded polymeric vehicle

Rheological properties

An aliquot of 10 ml of each formulation at different set speeds (1–8; Bohlin Visco 88 viscometer, UK) was used to determine the average (n = 3) of viscosity, and the rate of shear and shear stress at 25°C was used to identify the type of flow and the thixotropic properties (forward and backward curves were performed 60 s apart) (Figure 2).

Zeta potential

The electrokinetic stability of the metformin-loaded colloidal system was measured by Zetasizer Nano ZSP (Malvern Instruments, UK) in triplicates for all formulations. Few drops of each formulation were diluted in Milli-Q ultrapure water and placed in a suitable cuvette at 25°C and data were obtained via Zetasizer software. Results were recorded as mean ± SD (Figure 3).

Surface tension

The surface-active properties of different formulations (n = 3) were determined (Sigma 703 Tensiometer, Japan) by recording the force required to break a very thin-film formed inside a small ring. This small ring was immersed in a sample holder containing 30 ml of each formulation. The ring (attached to an upper hook) was slowly detached from the formulation surface by lowering the sample holder cup. All results were recorded as mean ± SD (Figure 3).

Characterization of prepared microcapsules

Particle size distribution of prepared microcapsules

The particle size distribution curve and size uniformity of freshly prepared microcapsules were determined by a Mastersizer 2000 (Malvern Instruments) that was operated by laser diffraction technology in detecting the hydrodynamic light-scattering behavior of spherical particles (Mie theory) [36,37]. Wet microcapsules were added to the sample dispersion unit, the laser was beamed into the sample and the average of triplicate measurements were automatically obtained by Malvern Mastersizer software. Results were recorded with respect to microcapsule median diameter, span and uniformity.

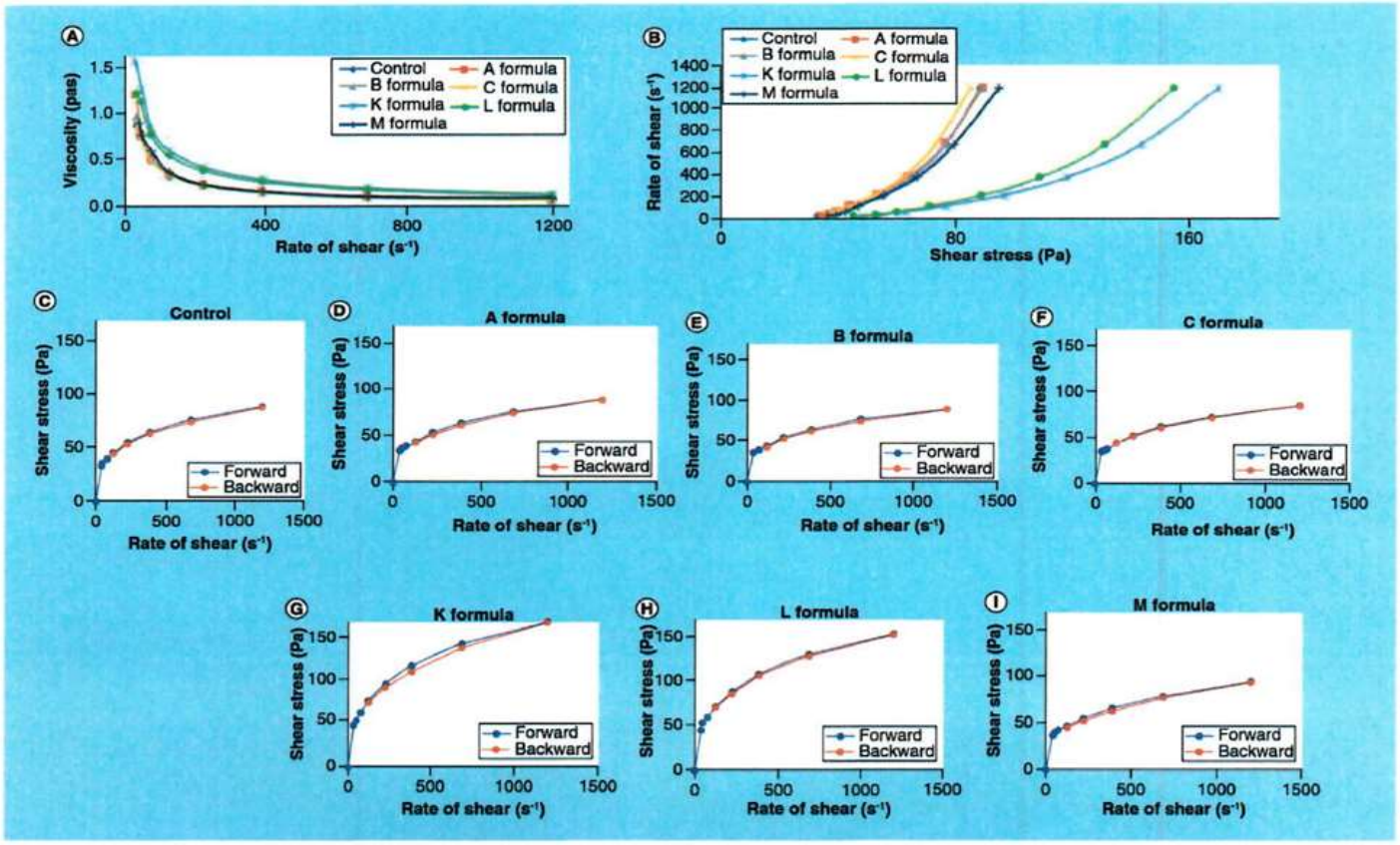

Figure 2. Rheological properties of control, A, B, C, K, L and M formulations. **(A)** Viscosity versus rate of shear curves, **(B)** rate of shear versus shear stress curves and **(C–I)** thixotropic rheograms representing shear stress versus rate of shear for **(C)** control formula, **(D)** A formula, **(E)** B formula, **(F)** C formula, **(G)** K formula, **(H)** L formula and **(I)** M formula.

Parameter/ instrument used / Formula	Viscosity (centipoise) at 25°C	Microcapsule diameter (µm)	Microcapsule median diameter (µm)	Span	Uniformity	Zeta potential (mv) at 25°C mean ± (SD) (n = 3)	Surface tension (mn/m) at 25°C mean ± (SD) (n = 3)
	Bohlin viscometer	Nikon optical microscope	(Malvern Mastersizer 2000, laser diffraction technology)			Malvern Zetasizer nano	Sigma 703 tensiometer
Control	880 (±0.81)	524.8 (±0.10)	657.43	1.103	0.358	-51.87 (±1.47)	59.41 (±0.006)
A	1210 (±0.80)	619.2 (±0.15)	726.89	0.784	0.246	-48.27 (±2.45)	58.70 (±0.015)
B	980 (±0.97)	580.3 (±0.15)	721.31	0.775	0.245	-50.40 (±2.71)	60.17 (±0.058)
C	890 (±0.96)	780.3 (±0.25)	810.56	0.784	0.248	-50.13 (±2.55)	60.68 (±0.020)
K	1600 (±0.72)	783.1 (±0.40)	794.89	0.698	0.221	-46.80 (±1.37)	57.71 (±0.006)
L	1230 (±0.79)	624.8 (±0.40)	741.71	0.676	0.209	-47.47 (±2.64)	58.71 (±0.010)
M	910 (±0.70)	524.8 (±0.75)	738.41	0.671	0.206	-50.93 (±1.36)	59.23 (±0.058)

Figure 3. Viscosity, zeta potential, surface tension of control A, B, C, K, L and M formula pre-encapsulation and optical microscopic images and mean diameter postencapsulation.

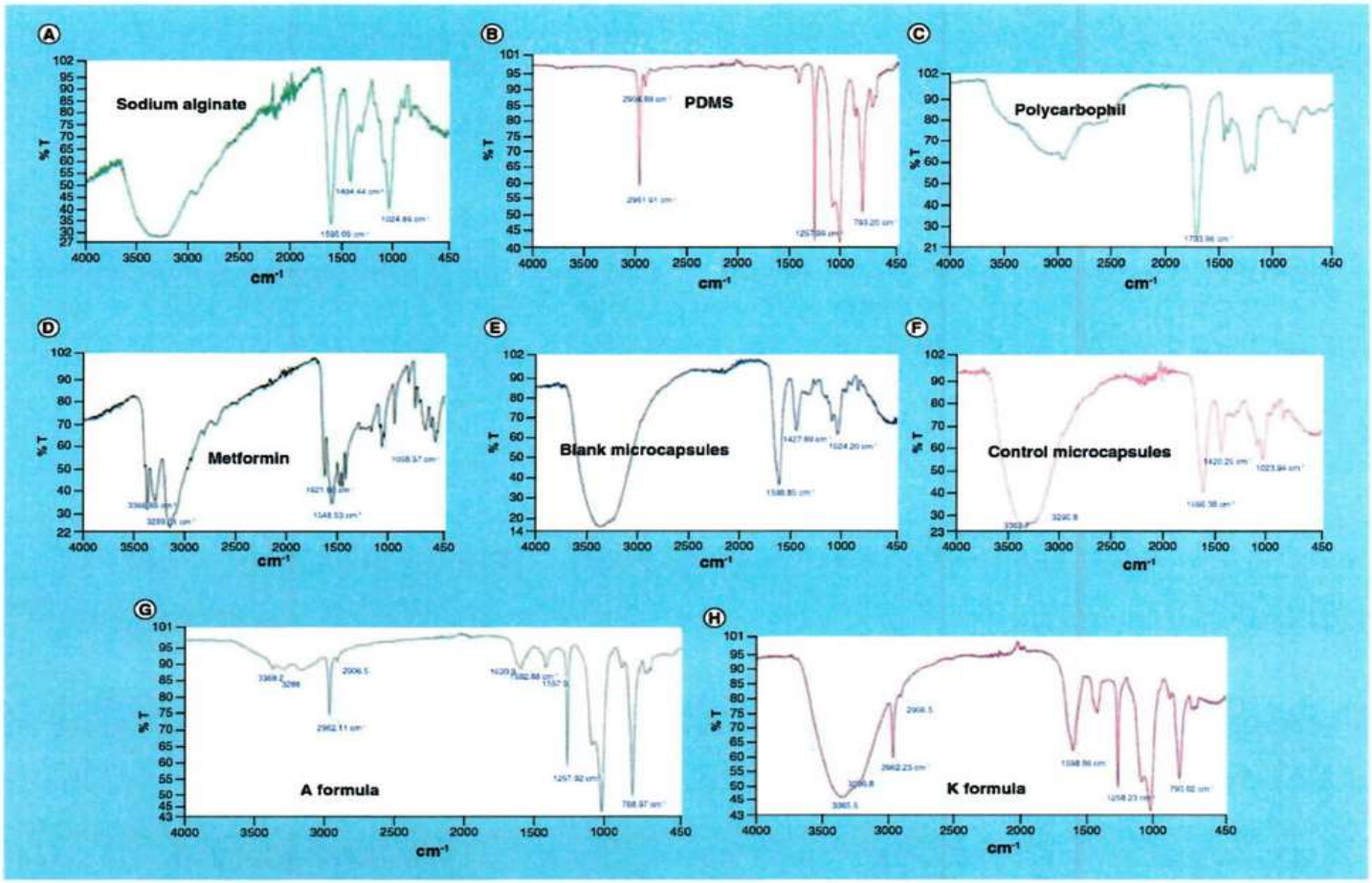

Figure 4. Chemical compatibility of metformin and the polymeric materials used in the produced microcapsules. FTIR charts for **(A)** sodium alginate, **(B)** PDMS, **(C)** polycarbophil, **(D)** metformin, **(E)** blank microcapsules, **(F)** control microcapsules, **(G)** A formula and **(H)** K formula.
FTIR: Fourier transform infrared; PDMS: Polydimethylsiloxane.

Optical microscopy

Microcapsule morphology of around ten microcapsules on a glass slide against a dark background was determined by Nikon SMZ800 stereo optical microscope (Nikon, USA) with a Toupcam 14 MPA camera, and the relevant microcapsule diameter of each formulation was measured by the connected Toupview software (Figure 3).

Fourier transform infrared spectroscopy

The Fourier transform infrared (FTIR) spectra of separate components (metformin, sodium alginate, PDMS and polycarbophil) as well as crushed microcapsule samples (control microcapsules, A formula, K formula and metformin-free blank microcapsules) were recorded in the scanning range between 4000 and 450 cm^{-1} at room temperature using a FTIR spectrometer (Perkin Elmer, USA) to check any possible interaction between metformin and the utilized polymers (Figure 4).

Production yield, drug loading & encapsulation efficiency

Production yield of every formulation can be calculated by determining the total weight of dried microcapsules and the total weight of metformin and polymers initially fed into the encapsulation process via Equation (1). Results were recorded as Mean ± SD (n = 3), Table 1.

$$\textit{Production Yield}\% = \frac{\text{total weight of dry microcapsules produced}}{\text{weight of metformin} + \text{total weight of polymers}} \times 100 \qquad \text{(Eq. 1)}$$

Metformin loading (drug content) and efficiency of the microencapsulation process were determined by placing an accurate amount of 100 mg of every formulation in a 200-ml volumetric flask and the final volume was made up to 200 ml using a phosphate buffer of pH 7.4. Triplicate determinations of all flasks were sonicated at 37°C for 30 min then placed on a multishaker PSU 20 (150 rpm, 37°C for 24 h) for complete disintegration. Samples were centrifuged for 5 min and the supernatant was then filtered via a syringe filter for HPLC analysis of the metformin concentration at 227 nm by a validated HPLC method, described elsewhere [38,39]. Metformin content was determined by calculating the weight of metformin contained in 100 mg of every formulation [32] as per Equation (2). Results were recorded as Mean ± SD (n = 3), Table 1.

$$\textit{Metforminloading}\,\% = \frac{\text{weight of metformin in sample microcapsules}}{\text{weight of sample microcapsules}} \times 100 \qquad \text{(Eq. 2)}$$

Microencapsulation efficiency was determined by calculating the practical and theoretical metformin content in 100 mg of every formulation [40] as per Equation (3). Results were recorded as Mean ± SD (n = 3), Table 1.

$$\textit{Microencapsulationefficiency}\,\% = \frac{\text{Practical weight of metformin}}{\text{Theoretical weight of metformin}} \times 100 \qquad \text{(Eq. 3)}$$

Carr's index & Hausner ratio

The flow properties of dry microcapsules were determined by measuring the initial volume occupied by 2 g of a formulation (Vi) and the volume occupied by the same weight after tapping a glass measuring cylinder 100-times on a bench, Tapped volume (Vt). Triplicate determinations of all formulations were recorded. Carr's index (compressibility index) and Hausner ratio [41] were calculated as per Equations (4 & 5). Results were recorded as Mean ± SD (n = 3), Table 1.

$$\textit{Carr's Index}\,\% = \frac{\text{Initial volume (Vi)} - \text{Tapped Volume (Vt)}}{\text{Initial volume (Vi)}} \times 100 \qquad \text{(Eq. 4)}$$

$$\textit{Hausner Ratio} = \frac{\text{Initial volume (Vi)}}{\text{Tapped volume (Vt)}} \qquad \text{(Eq. 5)}$$

Swelling studies

The swelling behavior of all formulations were assessed in 0.1 N HCl, pH 1.2 and in a phosphate buffer, pH 7.4 was contained in dissolution apparatus vessels. Volumetrically, 100 mg of every formulation was placed in a dissolution basket and immersed in 500 ml of each dissolution media (37°C, 50 rpm) using (Erweka DT6, Germany) dissolution apparatus. Swollen microcapsules were collected at predetermined intervals and weighed after drying the surface of microcapsules with paper towel to remove excess liquid droplets. All experiments were done in triplicates of all formulations. Swelling index (average, n = 3) was calculated by determining the correlation between the weight of dry microcapsules and swollen microcapsules [40] as per Equation (6). Swelling index % versus time was presented in Figure 5.

$$\textit{Swelling index}\,\% = \frac{\text{weight of swollen microcapsules} - \text{weight of dry microcapsules}}{\text{weight of dry microcapsules}} \times 100 \qquad \text{(Eq. 6)}$$

Mechanical strength/mechanical resistance

The mechanical stability of dry microcapsules to different solutions was tested. Ten microcapsules of each formulation were placed in 50-ml flasks of different media (distilled water, 0.9% NaCl solution and phosphate buffer pH 6.8). Triplicate flasks of every formulation in these tested media were placed on a multishaker PSU 20 that operated at 150 rpm, 37°C. The number of intact microcapsules were visually counted at predetermined time intervals. Average mechanical strength % (n = 3) [42] calculated as per Equation (7) versus time is shown in Figure 5.

$$\textit{Mechanicalstrength}\,\% = \frac{\text{number of intact microcapsules remained after shaking}}{\text{initial number of microcapsules}} \times 100 \qquad \text{(Eq. 7)}$$

Scanning electron microscopy & energy-dispersive x-ray

Few microcapsules were mounted using double-sided adhesive tape on a small round-glass stub and coated with platinum under a vacuum. The surface characteristics of dry microcapsules were examined by scanning electron

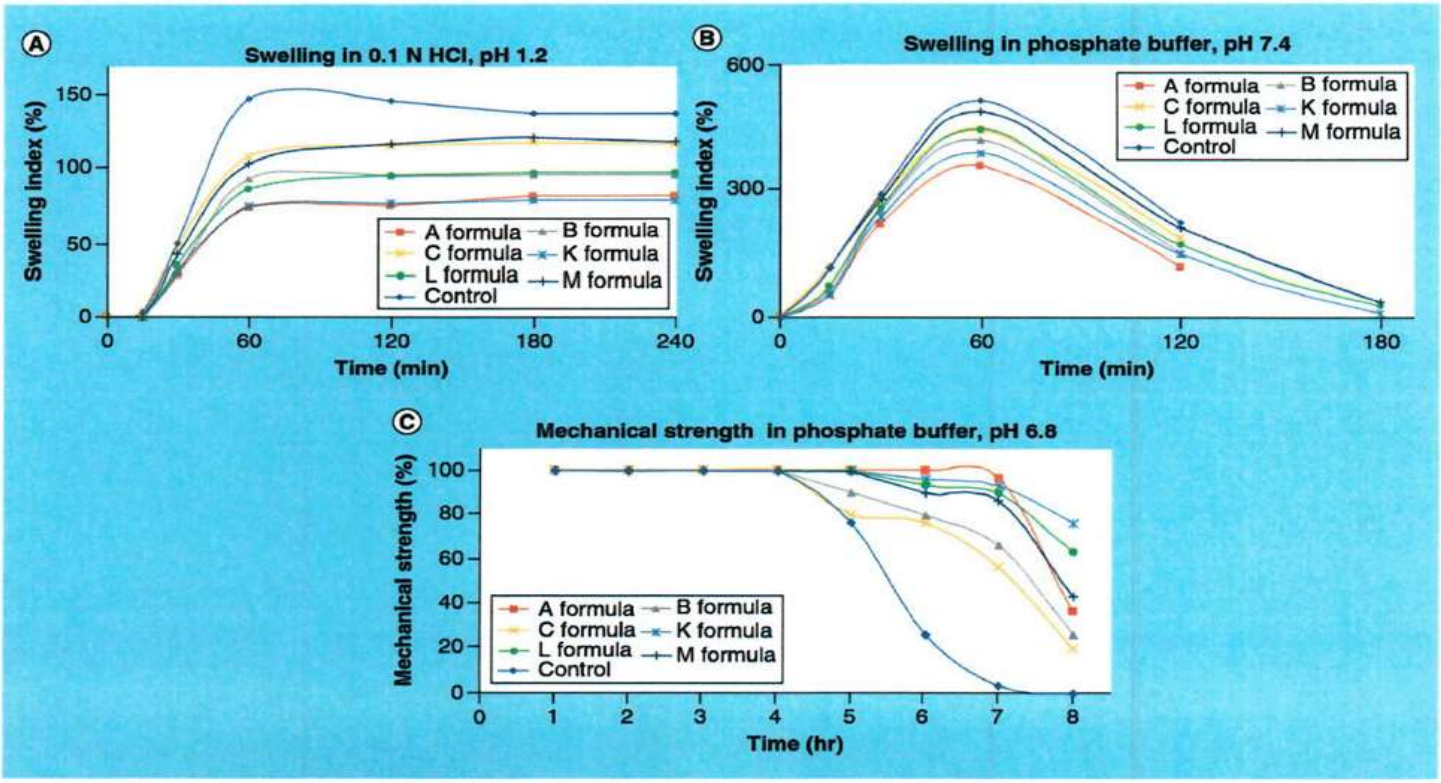

Figure 5. Swelling and mechanical strength curves of control, A, B, C, K, L and M formulations. (A) Swelling index versus time in 0.1 N HCl (pH 1.2), **(B)** swelling index versus time in phosphate buffer (pH 7.4) and **(C)** mechanical strength index versus time in phosphate buffer (pH 6.8).

microscopy (SEM; Tescan MIRA3 XMU, Czech Republic) operating at a 5-kV electron beam. Qualitative elemental analysis of microcapsules was performed with energy-dispersive x-ray (EDX; Oxford X-Max^N 150 SDD x-ray detector) and electron micrographs at suitable magnifications were recorded by Aztec software (Oxford Instruments, UK) to examine topographical features of all microcapsules.

Stability of the polymeric vehicle & the produced microcapsules

The zeta potential of metformin-loaded polymeric vehicles for all formulations was measured after 24 h of preparation at 25°C to provide an assessment of the stability of that colloidal system. Sample dry microcapsules of every formulation were wrapped twice in aluminum foil and kept in an accelerated stability chamber at 40°C and 60% relative humidity for 4 weeks. The physical stability of microcapsules was checked visually while chemical stability of metformin contained was determined by HPLC in triplicates [38,39], where 100 mg of every formulation were treated the same way as explained in Equation (2) in the section titled 'Production yield, drug loading and encapsulation efficiency' to determine metformin content after 4 weeks.

Results

Method development & optimization

The heterogeneous polymeric mixture of PDMS and sodium alginate was efficiently homogenized using an Ultra-Turrax homogenizer within 5 min and the new siliconized alginate platform was cross-linked with $CaCl_2$ to form microcapsules utilizing vibrational jet-flow ionic gelation (Figure 1).

Characterization of metformin-loaded polymeric mixture

Rheological behavior

All formulations showed decreased viscosity with an increased rate of shear applied in a nonlinear trend (Figure 2A). Another direct relationship between shear stress was applied on all formulations and the rate of shear was recorded and presented in Figure 2B. Figure 2A & B represents the non-Newtonian, shear-thinning properties of this polymeric platform. Thixotropy of the polymeric mixture was obvious in the small hysteresis loops (Figure 2C–I) between forward and backward rheograms of control, A, B, C, K, L and M formulations. K, L and M formulations exhibited higher viscosities than the A, B and C series (Figure 3).

Zeta potential

All formulations showed high electrokinetic stability (ranging from -46.8 to -50.93 mV) compared with -51.87 mV for the control (Figure 3).

Surface tension

The surface tension measured for the C platform was higher than that of B and A, which were the lowest in this category. The surface tension of polycarbophil formulations can be presented in the following order K < L < M. (Figure 3).

Characterization of microcapsules

Particle size distribution

All formulations showed good particle size distribution (narrow distribution curves, span <1 compared with control) with very good uniformity of particles (<0.4 deviation from the median size) (Figure 3).

Optical microscopy

Microcapsules produced from all formulations were spherical in shape with a smooth surface. These findings are in accordance with the Mastersizer results measured by laser diffraction technology (Figure 3).

Fourier transform infrared spectroscopy

No chemical incompatibility of metformin HCl, PDMS, sodium alginate and polycarbophil in the presented formulations was recorded (Figure 4). Control microcapsules (metformin-loaded calcium alginate) (Figure 4F), A formula (Figure 4G) and K formula (Figure 4H) (the highest polymeric content formulation) did not show any substantial shifting of absorption peaks of metformin HCl (Figure 4D) or the used polymers (Figure 4A–C).

Production yield, drug loading & encapsulation efficiency

All formulations showed high production yield more than 97.7% (Table 1). They also showed much higher metformin loading and encapsulation efficiency than the control (Table 1).

Flow properties of dry microcapsules

All Buchi-encapsulated formulations including the control showed good flow characteristics where Hausner's ratio was in the range 1.12 to 1.18 and Carr's Index was in the range 11 to 15 as per United States Pharmacopeia (USP 37) specifications.

Swelling studies

Generally, all formulations and controls showed less swelling in HCl compared with the phosphate buffer. Formulations with higher PDMS content (A and K) had much smaller swelling indices due to their hydrophobic nature toward aqueous uptake from both media, despite complete wetting of their surfaces (Figure 5A & B).

Microcapsule integrity & mechanical strength

Microcapsules of all formulations and control remained intact for 24 h in distilled water (slightly swollen). In normal saline, only control microcapsules showed 80% mechanical strength index, where 20% of control microcapsules experienced full rupture after 24 h, while other formulations remained intact (data not shown). After 8 h in a phosphate buffer pH 6.8, the mechanical indices of PDMS formulations can be presented in the order A > B > C (Figure 5C). Incorporation of polycarbophil improved the overall mechanical strength/microcapsule integrity.

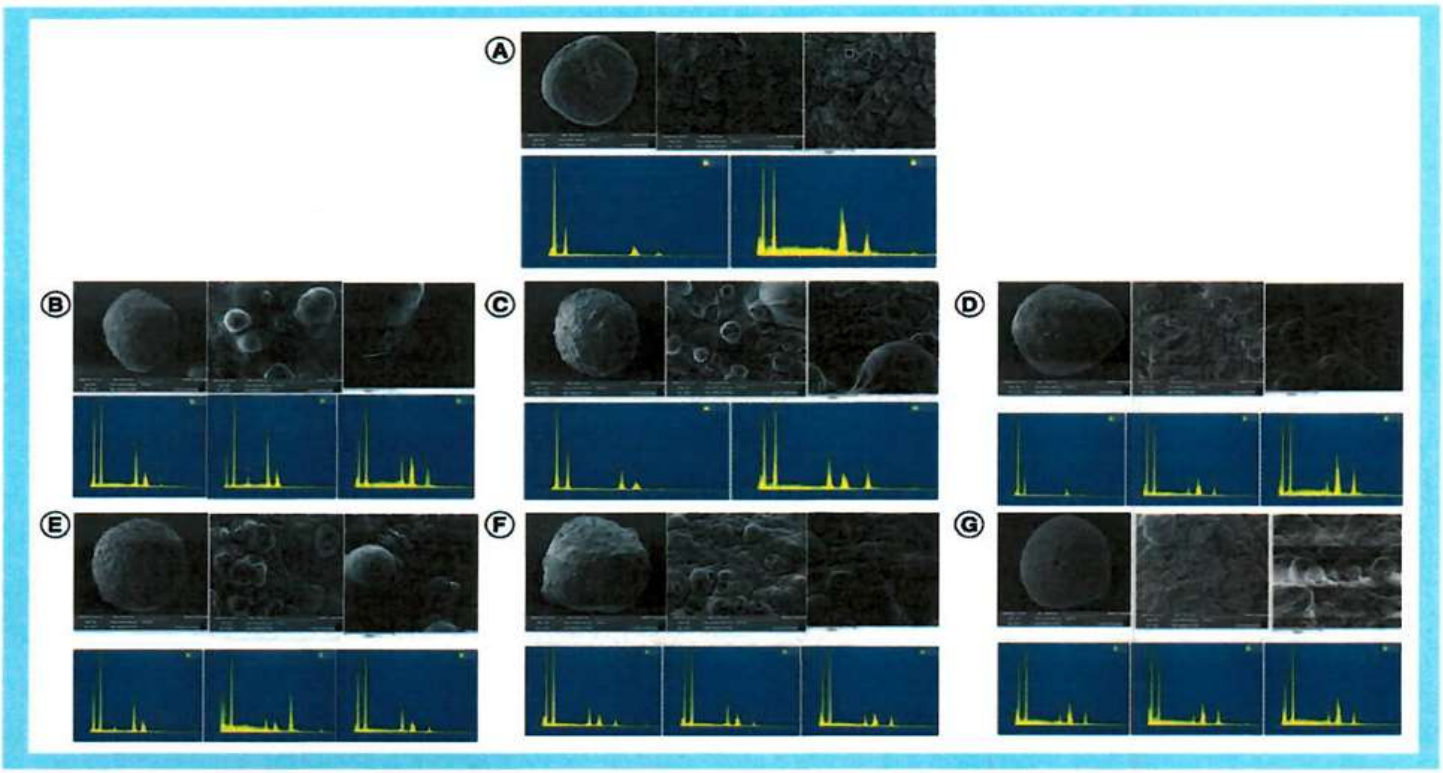

Figure 6. Scanning electron microscopy and energy-dispersive x-ray of microcapsules. (A) Control, **(B)** A formula, **(C)** B formula, **(D)** C formula, **(E)** K formula, **(F)** L formula and **(G)** M formula.

The order of mechanical strength of all formulations at the end of 8 h can be presented as follows K > L > M > A > B > C > control.

Scanning electron microscopy & EDS

SEM images of dry microcapsules of all formulations revealed that they are opaque, discrete, well-defined spheres (in accordance with optical microscopic findings), and completely covered with a nonporous continuous polymeric coat. EDS showed an even distribution of Silicon atoms (Si) and carbon atoms (C) on different sites of the microcapsule surface, which reflects a continuous PDMS/alginate coat (Figure 6).

Stability of polymeric platform & the produced microcapsules

The PDMS-grafted alginate platform loaded with metformin showed good electrokinetic stability after 24 h at room temperature and no significant change in zeta potential of any formulations was recorded (data not shown). After 4 weeks in the accelerated stability chamber (40°C and 60% RH), all microcapsules and the control were darker in color and no differences were observed macroscopically in morphology or size of all microcapsules. All formulations and controls retained the original content of metformin after 4 weeks with no significant difference in drug contents within the microcapsules (Supplementary Figures 1–7).

Discussion

Due to the low density and oily nature of PDMS, it could not be directly used to encapsulate pharmaceuticals before it got cross-linked, emulsified or both. PDMS is immiscible with a sodium alginate solution and early trials to mix PDMS with sodium alginate (different grades and different viscosities) using a magnetic stirrer (maximum speed, 24 h) or high-speed mixer (2000 rpm, 3–5 h) were unsatisfactory. The produced emulsion did not show satisfactory stability, and elongation of the mixing time produced too viscous dispersions that were unamenable to processing via Buchi B-390 for encapsulation, especially after metformin addition. PDMS was solubilized in a small volume of dichloromethane (a water-miscible and volatile organic solvent), then water and medium viscosity sodium alginate

powder were added to the organic layer. The polymeric blend was then homogenized (20,000 rpm) for four cycles (1 min each, 30 s apart) to obtain a homogeneous polymeric dispersion. Metformin was then added, and the entire system was homogenized for 1 more minute. Polycarbophil (suspending agent and emulsion stabilizer) was added to some formulations to study their effect on electrokinetic stability, size, drug loading, encapsulation efficiency and flow properties of microcapsules. This technique self emulsifies PDMS by alginate grafting to form a stable heterogenous polymeric platform and cross-link the aqueous terminals of the produced amphiphilic dispersion by a vibrating jet-nozzle ionotropic gelation using gently agitated $CaCl_2$ as a cross-linker to produce free-flowing microcapsules.

Understanding the rheological behavior in a new pharmaceutical platform is quite important to estimate the stability, drug absorption and the bioavailability of a given therapeutic [43]. Non-Newtonian flow is quite common not only in pharmaceutical products but in many industries and biologicals as well [43,44]. All formulations including the control exhibited non-Newtonian, pseudoplastic (shear-thinning) flow. The increase in the rate of shear applied was accompanied by a noticeable decrease of polymeric resistance to flow (viscosity), due to changes occurring in the intermolecular forces between the polymeric blocks within each formula [45]. Formulations also exhibited thixotropic behavior characterized by small hysteresis loops generated between forward and backward rheograms (60 s apart). The new polymeric platform of all formulations showed slow recovery of their original consistency upon a reduction of stress applied. The viscosity measured at the same speed point (at 25°C) for all formulations, showed that the control sample had the lowest viscosity of all and the higher the PDMS content, the higher the viscosity. The order of viscosities can be presented in the following order (A > B > C). Polycarbophil formulations showed much higher viscosities compared with polycarbophil-free counterparts in the following order K > L > M. The higher viscosity of K, L and M could be attributed to the viscosity-imparting nature of polycarbophil.

The electric potential measured in the interfacial double layer of the metformin-loaded polymeric dispersion was used to evaluate the degree of stability of the colloidal polymeric vehicle. A higher value of zeta potential indicated higher repulsion between similarly charged particles within the system, hence the higher stability of such colloidal dispersion and the uniform distribution of metformin among different polymeric molecules of PDMS and alginate. It was reported that there is an inverse relationship between surface tension and the wetting of particles [46], where the higher the surface tension the harder it was to wet coated particles [46]. The interfacial tension measured between liquid phases, knowing their rheological properties enables the formation of stable dispersed systems [47]. These data shall give an idea about the initiation of the encapsulation process and predict the final capsule morphology [47]. We noticed that PDMS decreased the surface tension of the system (compared with control) and the higher the PDMS content, the lower the surface tension of the formula. Polycarbophil addition further reduced the surface tension with respect to PDMS content in the K, L and M series. Based on these results, A and K formulations would have relatively higher surface wettability. Such an effect, to some extent along with rheological properties, viscosity and drug content might have an impact on the produced microcapsule size when exposed to a cross-linker solution of $CaCl_2$, where the K formula was expected to have a higher wettability, showed the largest diameter of all formulations.

The data collected from distribution curves of microcapsules verified the precision and efficiency of vibrational jet-flow technology and Buchi encapsulation of metformin in the new polymeric coats. Polycarbophil addition improved the uniformity and the span of K, L and M compared with A, B, C and control. We have noticed a relationship between the formulation viscosity and the microcapsule size produced, where microcapsule size/diameter decreased as the viscosity decreased. Formula C was an exception to that trend, and although it has lower viscosity than A and B, it showed larger microcapsule size than A and B. This could be explained by the fact that C has the smallest amount of metformin in the A, B and C series, hence less ionic interaction between the positively charged amino groups of the biguanide and the negatively charged carboxylate groups of alginate, bearing in mind the neutral (uncharged) terminals of PDMS (compared with higher metformin content and hence higher ionic interaction in Formula A and B). Formula M, on the other hand, has the smallest microcapsule size of all formulations, and despite the lowest metformin content in the K, L and M series, the M formula followed the size/viscosity relationship and did not behave like the C formula. This could be attributed to a higher ionic interaction of amino groups of its metformin content with carboxylate terminals of not only alginates but with the (-COO-rich) polycarbophil as well.

Drug–polymer interaction might be a limitation while developing new therapeutic vehicles, as it could affect drug properties and sometimes the pharmacological effect. Drug–polymer interaction can be verified through FTIR spectroscopic analysis. The metformin HCl spectrum showed absorption peaks at 3366 cm^{-1} (-NH symmetric

stretching), 3289 cm^{-1} (asymmetric -NH stretching), 1621 cm^{-1} (C = N stretching vibration), 1548 and 1058 cm^{-1} (N–H bending and C–N stretching of amines, respectively) (Figure 4D) [48]. The absorption peaks for the spectrum of PDMS were at 2961 cm^{-1} (-Si $(CH_3)_2$-O-Si $(CH_3)_2$-), 2905 cm^{-1} (-Si $(CH_3)_3$), 1258 cm^{-1} (Si $(CH_3)_2$) and 793 cm^{-1} (-Si-CH_3-) (Figure 4B) [26]. Characteristic sodium alginate peaks appeared at 1595 cm^{-1} (asymmetric -COO- stretching), 1404 cm^{-1} (symmetric -COO- stretching) and 1024 cm^{-1} (C-O-C) stretching vibration (Figure 4A) [32]. Polycarbophil showed only one sharp peak of (C = O of carboxylates) at 1704 cm^{-1} (Figure 4C). The FTIR spectra of blank microcapsules showed slight shifting of (symmetric -COO-) peak from 1404 to 1427 cm^{-1} (due to ionic gelation of Na-alginate and conversion into Ca-alginate salt) (Figure 4E). Metformin-loaded microcapsules (Figure 4F), the A formula (Figure 4G) and the K formula (Figure 4H) did not show any significant changes to metformin peaks.

Compared with the control, PDMS had a positive impact on metformin loading and encapsulation efficiency. The higher the PDMS content, the higher the metformin loading of the formula and its encapsulation efficiency. In the A, B and C series, A formula had the highest drug loading 5.99% (±0.13) and the highest encapsulation efficiency 17.99% (±0.39). This could be explained by the fact that the hydrophobic nature of PDMS could have acted as a barrier coat against the escape of the water-soluble metformin during the ionotropic gelation process. The addition of polycarbophil slightly improved both drug loading and encapsulation efficiency in the K, L and M formulations. The order of metformin loading and encapsulation efficiency for polycarbophil formulations can be presented as follows K > L > M.

Good-flow properties are required in some pharmacopeias such as USP and European Pharmacopeia to ensure accurate delivery of drugs from dosage forms [35]. All prepared formulations showed good-flow properties in terms of Hausner's ratio and Carr's index as per USP.

The swelling properties of the formulated microcapsules are mainly attributed to the water uptake tendency of sodium alginate to the surrounding aqueous media, where PDMS is lipophilic in nature and would resist swelling of formulations in both HCl and the phosphate buffer. Maximum acid swelling was noticed within 60 min after which no swelling was noticed for 4 h in the acidic media, due to shrinkage of alginate blocks at low pH for all formulations [33]. In the phosphate buffer, an ion exchange between the cross-linked divalent calcium ions (Ca^{2+}) of microcapsules and the monovalent sodium ions (Na^{+}) present in the buffer media took place. Such ionic exchange is thought to be the main reason for loosening of the polymeric backbone and within 60 min a significant swelling at pH 7.4 was noticed, after which polymeric matrix started to erode. Slight swelling of the control and all formulations was reported after 24 h in distilled water due to minimal water uptake of sodium alginate in distilled water, however more aqueous uptake was noticed in 0.9% NaCl, which caused degradation or rupture of only the control sample. Formulations A, B, C, K, L and M showed an ability to withstand the normal saline mechanical strength and no rupture was recorded in any of them. The hydrophobic effect of PDMS enabled the presented formulations to undergo minimal uptake of the phosphate buffer pH 6.8 and hence improved microcapsule integrity and mechanical resistance toward this buffer over 8 h, where formulations with higher PDMS ratio exhibited better mechanical strength. Polycarbophil, on the other hand, augmented the mechanical properties with respect to the PDMS ratio. The K formulation produced with both polycarbophil and higher PDMS ratio had the highest mechanical strength of all the formulations.

SEM images of dry control microcapsules showed a rough wrinkled surface with several channels or cracks possibly created upon drying of the alginate network. Surface composition by EDS showed variable levels of nitrogen atoms (N) at different sites, which reflects migration of metformin to the surface of control microcapsules. All formulations showed better spherical shapes than the control after drying, probably due to elasticity imparted by PDMS. Multiple bulges on the surface of A, B, K and L could be attributed to partial dissociation of PDMS globules from the polymeric matrix post-drying and -vacuuming of these formulations for the electron microscopic (EM) imaging process. These bulges are in fact relevant to PDMS content where minimal bulges were noticed in C and M (with the lowest PDMS ratio) and their surface looked similar to the control without cracks or channels. Polycarbophil addition improved the surface appearance of microcapsules of K, and L compared with A and B (less bulging of PDMS globules was noticed in K and L). Metformin appeared on the surface of most microcapsule formulations (identified by variable levels of nitrogen atoms at different surface sites, characteristic feature of the biguanide). Interestingly, Formula A did not show any metformin on the surface, which could be attributed to the most hydrophobic surface it possesses (the highest PDMS ratio and no polycarbophil). Such surface features of Formula A could have hindered or diminished by surface disposition of the hydrophilic metformin or even

limited by the internal migration of metformin to the microcapsule surface. Small crystals of NaCl identified by Na atoms were also detected on the surface of some microcapsules, formed as a byproduct of a Na-alginate and $CaCl_2$ ionotropic gelation reaction.

The new silicon-grafted-alginate platform loaded with metformin reserved its good electrokinetic stability at room temperature over 24 h. Microcapsules of all formulations, after 4 weeks in the accelerated stability chamber (40°C and 60% relative humidity [RH]), were darker in color (brownish yellow), potentially due to oxidation [49–51] without significant size changes. HPLC analysis of all formulations and the control after 4 weeks in the aforementioned stress conditions revealed that these microcapsules have the potential to preserve the metformin content for the specified period (Supplementary Figures 1–7) and no degradation was identified on any of the supplemented chromatograms.

It has been reported that drug release characteristics from microcapsules are governed by different variables such as drug properties, formulation technique, surrounding release media, nature and physicochemical properties of the polymeric vehicle used [35]. In fact, characterization of a newly developed polymeric platform plays a pivotal role in the prediction of the drug release process [35]. In our case, the water-soluble metformin is not expected to diffuse through the hydrophobic PDMS terminals, although diffusion through sodium alginate blocks is quite possible. Water uptake and the swelling ability of sodium alginate were reported to be high (more than 200-times its original weight) in a pH-dependent manner [35]. PDMS, on the other hand, was hindered to some extent with extensive swelling of alginate. The swelling characteristics were much higher in the control microcapsules compared with PDMS formulations in both HCl (pH 1.2) and the phosphate buffer (pH 7.4), yet the swelling in the phosphate buffer was almost four-times than that in HCl. The aqueous uptake and the swelling properties could have participated in the mechanical properties of the developed microcapsules, where the control showed the lowest mechanical strength of all microcapsules in distilled water, normal saline and the phosphate buffer pH 6.8. Despite the swelling ability of polycarbophil at pH 6.8, it rather improved the microcapsule integrity in terms of the mechanical strength, due to its emulsifying and stabilizing propertied in the presented polymeric blend. In fact, the new PDMS-grafted-alginate offered a noticeable improvement in metformin loading and encapsulation efficiency of the silicon-based microcapsules compared with the control. However, these criteria are still to undergo meaningful release studies, where metformin could initially be released but below an instrumental detection limit. This could represent a limitation of the new microcapsules, but the data collected from rheological properties of the polymeric blend, swelling behavior and the mechanical resistance of these microcapsules will enable us to design optimal conditions to improve the drug loading and encapsulation efficiency, to effect drug release *in vitro* and *in vivo* in future.

Conclusion

A therapeutic platform for pharmaceutical delivery was investigated. The metformin-loaded polymeric vehicle displayed pseudoplastic, thixotropic, rheological properties with good electrokinetic stability. No chemical interaction was detected between metformin and the polymers used. The microcapsules produced by the ionic gelation vibrational jet-flow technology had uniform particle size distribution and all microcapsules were free-flowing spheres. These microcapsules were also evaluated for their swelling and mechanical resistance in different pH media and have shown suitable characteristics appropriate for drug stability and delivery. Our future work will focus on optimizing the drug load, the encapsulation efficiency and study the release characteristics of the optimized new silicon-based formulations.

Future perspective

In the proposed polymeric vehicle, we have created a platform for drug delivery based on a PDMS polymer, which has shown promise in the formulation of nano- and micro-delivery matrices, with good stability data. However, due to its physicochemical characteristics, PDMS-customized drug delivery systems remain challenging in terms of drug encapsulation efficiency and content. Further scale-up techniques as well as drug–PDMS ratio testing are needed in order to optimize the large-scale production of efficient microcapsules that are capable of forming a wide range of drug delivery matrices, that includes different pharmaceuticals or biological agents such as genes, cell lines, microorganisms, macromolecules, enzymes and antibodies. These silicone particulates can be used orally or targeted to certain tissues or organs, such as transplantable or injectable therapeutics. Accordingly, moving forward, PDMS-customized therapeutic delivery has a significant potential and wide applications in the drug and biological delivery of therapeutics.

Summary points

- A polydimethylsiloxane (PDMS)-grafted-alginate delivery vehicle was developed and tested.
- The new therapeutic delivery platform has pseudoplastic, thixotropic, rheological properties with good electrokinetic stability profiling.
- The new siliconized microcapsules have uniform size distribution with good flow properties.
- Good compatibility between metformin and the utilized polymers in the encapsulation process is reported in this study.
- PDMS-based formulations have higher metformin content and entrapment efficiency than the control.
- The new PDMS-customized microcapsules offer good stability of the contained metformin after 4 weeks in accelerated stability conditions.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.future-science.com/doi/suppl/10.4155/tde-2020-0049

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Chapter 3

Graphical abstract

Chapter 3: Development and validation of a new analytical HPLC method for simultaneous determination of the antidiabetic drugs, metformin and gliclazide

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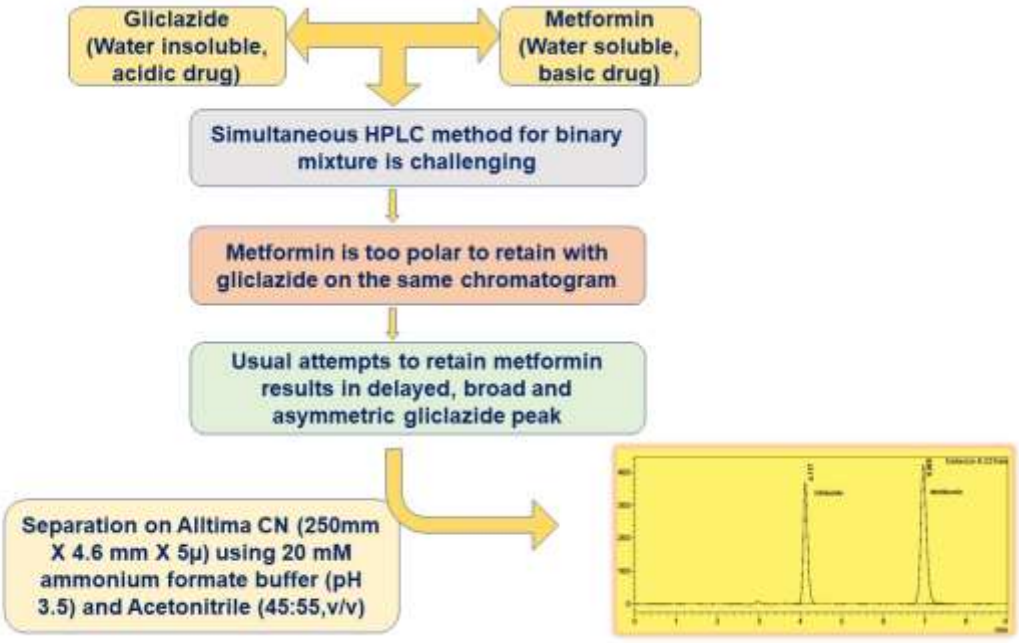

This chapter outlines the development and validation of a new analytical HPLC method for the simultaneous analysis of the water-soluble, basic biguanide (metformin) and the water-insoluble, acidic sulfonylurea (gliclazide). This new method was applied for the simultaneous analysis of marketed tablets of metformin and gliclazide.

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Original Article

Development and validation of a new analytical HPLC method for simultaneous determination of the antidiabetic drugs, metformin and gliclazide

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ABSTRACT

An efficient and simple HPLC method has been developed and validated for the simultaneous determination of gliclazide and metformin hydrochloride in bulk and was applied on marketed metformin and gliclazide products. The mobile phase used for the chromatographic runs consisted of 20 mM ammonium formate buffer (pH 3.5) and acetonitrile (45:55, v/v) The separation was achieved on an Alltima CN (250 mm × 4.6 mm x5μ) column using isocratic mode. Drug peaks were well separated and were detected by a UV detector at 227 nm. The method was linear at the concentration range 1.25–150 μg/ml for gliclazide and 2.5–150 μg/ml for metformin respectively. The method has been validated according to ICH guidelines with respect to system suitability, specificity, precision, accuracy and robustness. Metformin limit of detection (LOD) and limit of quantification (LOQ) were 0.8 μg/ml and 2.45 μg/ml respectively while LOD and LOQ for gliclazide were 0.97 μg/ml and 2.95 μg/ml respectively.

1. Introduction

Metformin is an effective biguanide antidiabetic agent that has been used to control blood glucose level of type II diabetic patients for decades and has been considered the first line treatment according to international guidelines [1,2]. Mitochondrial inhibition and activation of AMPK are key molecular effects of metformin to inhibit hepatic gluconeogenesis [1,2]. Metformin on the other hand can directly and indirectly improve skeletal muscle sensitivity towards insulin [3].

Gliclazide is a second-generation sulfonylurea which binds to a specific sulfonylurea receptor on the pancreatic β-cells to enhance insulin secretions [3,4]. In addition to its pancreatic effects, Gliclazide can play a significant role in the treatment of diabetic vascular disease through its antioxidant properties [5].

There are several HPLC methods either in pharmaceutical products or biological samples reported in the literature for determination of metformin alone [6,7], gliclazide alone [8,9], metformin with other agents [10–13], gliclazide with other therapeutics [14,15] or metformin and gliclazide together

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[16,17]. Some of these methods used gradient elution to separate the tested analytes [10] [18]. Literature also reveals the use of ion pairing technique [18] [19–21] and micellar liquid chromatography [22] to develop a successful HPLC method for the determination of gliclazide and/or metformin. The reported LODs of metformin in some literature were quite high 12 μg/ml [21], 22.93 μg/ml [23] and the reported sensitivity of other methods in terms of LODs for gliclazide were 1.19 μg/ml [15] and 3.9 μg/ml [24].

The present study is aiming to develop and validate a simple, sensitive, rapid, economic and isocratic HPLC method for the determination of both metformin and gliclazide on the same chromatographic run without the need for derivatization or precolumn treatment.

2. Experimental

2.1. Materials, reagents and pharmaceutical products

Gliclazide >98%, metformin hydrochloride 97% and analytical reagent grade ammonium formate were purchased from Sigma–Aldrich (St. Louis, USA). Metformin® (metformin 500 mg tablets, Sandoz) (Sydney, Australia) and Glyade® (gliclazide 80 mg tablets, Alphapharm) (Brisbane, Australia) were obtained from local pharmacy. Analytical reagent grade formic acid was obtained from Ajax Fine Chem Pty Ltd (Melbourne, Australia). HPLC grade acetonitrile was procured from Fisher Chemical, Thermo Fisher scientific (Melbourne, Australia). A Millipore Milli-Q water ultra-pure water system (Millipore, Australia) was used to obtain distilled water.

2.2. Instrumentation

The HPLC system used for the method development and validation consisted of Shimadzu, Japan equipped with a LC-20AT pump with inline degasser, SPD-20A $_{SR}$ UV detector and SIL-20AC $_{HT}$ Autosampler. Data acquisition, recording and chromatographic integration was performed by Labsolutions version 5.82. Analysis and separation has been done on an Alltima CN column 250 mm × 4.6 mm x 5μ at 227 nm in an air-conditioned lab (temperature maintained at 25 °C throughout all chromatographic runs). The mobile phase consisted of ammonium formate buffer (20 mM), pH 3.5 and acetonitrile at ratio (45:55, v/v), the flow rate was set at 1 ml/min in an isocratic mode and the injection volume was set at 20 μl for all samples.

2.3. Preparation of the buffer solution

A 20 mM buffer solution was prepared by dissolving 1.26 g ammonium formate in 1000 mL Milli-Q water and the final pH adjusted to 3.5 using formic acid. The buffer solution was then filtered through (0.45 Nylon NY membrane filter) and degassed in a sonicator for 10 min.

2.4. Preparation of standard stock solutions (A and B)

25 mg of metformin was accurately weighed and transferred into 100 ml volumetric flask and 20 mL of the mobile phase mixture was added to metformin and sonicated for 10 min, the final volume was made up to 100 mL using the mobile phase mixture (Flask A). In a separate volumetric flask, the same procedure was followed to dissolve 25 mg gliclazide (Flask B).

2.5. Preparation of working solution (mixture)

An aliquot of 2 mls from flask A and 2 mls from flask B were transferred into 10 ml volumetric flask and the final volume was made up with the mobile phase to give a working solution of metformin (50 μg/ml) and gliclazide (50 μg/ml).

2.6. Preparation of pharmaceutical samples

20 tablets of Metformin® Sandoz were weighed and crushed. 583.35 mg powder equivalent to one Metformin® tablet (500 mg metformin) was placed in a 500 ml volumetric flask (Flask C), 20 tablets of Glyade® Alphapharm were weighed and crushed the same way and 157.3 mg powder equivalent to one Glyade® tablet (80 mg gliclazide) was transferred into a 200 ml volumetric flask (Flask D). 10 mls of distilled water were added to (Flask C) and (Flask D) and sonicated for 10 min and the final volume was made up to the mark of both Flask C and D with mobile phase mixture followed by 5 min shaking. Flask C and D were filtered and separately 10 mls of the filtrate from each flask were transferred into two separate 20 ml volumetric flasks (Flask C and Flask D) and final volume was made to the mark with the mobile phase mixture. An aliquot of 2 mls from Flask C and 5 mls from Flask D were transferred into two separate 20 ml volumetric flasks and mobile phase was added to the mark to produce a final concentration of 50 μg/ml metformin flask and 50 μg/ml gliclazide flask respectively.

2.7. Method development and optimisation

Due to the significant difference in the physical and chemical properties of metformin and gliclazide, several mobile phases and columns were initially trialed in order to have both eluents on the same chromatogram. The suitability of the column and the mobile phase used in the optimized method have been decided based upon the basis of the selectivity, sensitivity as well as acceptable chromatographic parameters of the produced peaks in terms of peak sharpness, peak symmetry, tailing factor and resolution between the two peaks. We used the mobile phase as a solvent for all samples to ensure minimum noise and to eliminate any unwanted solvent peaks.

2.7.1. Columns applied in our initial trials

- Apollo C18 (150 mm × 4.6 mm x 5μ).
- Phenomenex Luna C18 (150 mm × 4.6 mm x 5μ).
- Phenomenex Luna C18 (100 mm × 2 mm x 5μ).
- Phenomenex Jupiter C18 (250 mm × 4.6 mm x 5μ).
- Alltima HP CN (150 mm × 4.6 mm x 3μ).
- Alltima CN (250 mm × 4.6 mm x 5μ) for the optimised method

2.7.2. *Examples of buffers trialed either with methanol or acetonitrile, based on previous literature*

- Phosphate buffer, different pH values 2.5, 3, 3.5, 5.3,6 and 7.3.
- Acetate buffer pH 3.
- Formic acid 0.05%.
- Ammonium formate for the optimised method.

2.7.3. *Selection of UV wavelength*

Gliclazide has a λ_{max} at 228 nm and metformin has λ_{max} at 234 nm in a water and methanol mixture (60:40) [25]. An acceptable response was obtained upon detection of both drugs at 227 nm either individually or in combination.

2.8. *Method validation*

The optimized method for simultaneous determination of metformin and gliclazide has been validated as per International Conference of Harmonisation (ICH) guidelines Q2 (R1) [26] for evaluating system suitability, specificity, precision, accuracy, linearity, limit of detection (LOD), limit of quantitation (LOQ) and robustness.

2.8.1. *System suitability*

System suitability parameters with respect to tailing factor, repeatability, number of theoretical plates and resolution between metformin and gliclazide peaks were assessed by injecting a blank mobile phase followed by six replicates of metformin (50 μg/mL)/gliclazide (50 μg/ml) mixture.

2.8.2. *Precision, repeatability (intra-day precision) and intermediate precision (inter-day precision)*

System and method precision were assessed by injecting 6 independent combined samples of metformin and gliclazide (50 μg/ml each) on the same day under same operating conditions.

Intermediate or inter-day precision was assessed by comparing the results of 6 independent determinations on 3 different days.

2.8.3. *Specificity/selectivity*

The effect of excipients commonly used in our tableting laboratory was checked, where (placebo matrix) was composed of anhydrous lactose NF, Letco medical (Decatur, USA), microcrystalline cellulose NF (Avicel), Letco medical (Decatur, USA), polyvinylpyrrolidone (PVP), Merck Pty Ltd (Melbourne, Australia), Potato starch, Fisher Scientific (Loughborough, UK), and magnesium stearate, BDH laboratories (Poole, UK). The specificity of the proposed HPLC method for the determination of metformin and gliclazide has been established by injecting the mobile phase, placebo matrix extracted solution and the pharmaceutical products (Metformin® Sandoz and Glyade® Alphapharm) into the HPLC system.

2.8.4. *Linearity and range*

Flask A, the standard stock solution of metformin is diluted in the concentration range of (2.5–150 μg/ml). Triplicates of such concentration range were prepared and plotted on a metformin calibration curve.

Flask B, the standard stock solution of gliclazide is diluted in the concentration range of (1.25–150 μg/ml). Triplicates of

Table 1 – System suitability and precision results (acceptance limit RSD % < 2).

	Retention time		Tailing factor		Number of theoretical plates		Resolution
	gliclazide	metformin	gliclazide	metformin	gliclazide	metformin	
1	4.117	6.965	1.226	1.166	6827	11,354	12.365
2	4.108	6.965	1.224	1.165	6857	11,399	12.437
3	4.103	6.963	1.23	1.163	6854	11,467	12.482
4	4.098	6.964	1.223	1.159	6888	11,475	12.525
5	4.093	6.964	1.224	1.156	6881	11,506	12.563
6	4.091	6.965	1.23	1.157	6904	11,525	12.591
Mean	4.101	6.964	1.23	1.161	6868.5	11,454.3	12.49
Standard deviation	0.009	0.0008	0.003	0.004	27.79	65.39	0.084
RSD%	0.239%	0.012%	0.255%	0.365%	0.405%	0.571%	0.671%

Precision results as peak area of different determinations on 3 different days, (metformin 50 μg/ml, gliclazide 50 μg/ml) (n = 6), acceptance limit RSD% <2)

	Day 1		Day 2		Day 3	
	gliclazide	metformin	gliclazide	metformin	gliclazide	metformin
1	2,657,308	4,169,612	2,584,188	4,165,847	2,632,224	4,614,954
2	2,646,317	4,134,736	2,588,337	4,214,888	2,611,676	4,624,234
3	2,641,722	4,133,151	2,587,760	4,159,595	2,625,581	4,650,749
4	2,625,888	4,128,304	2,615,001	4,176,160	2,627,764	4,621,892
5	2,636,926	4,128,241	2,597,393	4,184,036	2,634,300	4,593,732
6	2,631,707	4,110,705	2,596,967	4,207,524	2,648,357	4,600,653
Mean	2,639,978	4,134,124.8	2,594,941	4,184,675	2,629,983.667	4,617,702.333
Standard deviation	11,133.78	19,378.60	11,165.36	22,321.80	12005.91735	20,125.14913
RSD%	0.422%	0.469%	0.430%	0.533%	0.457%	0.436%

such concentration range were prepared and plotted on a gliclazide calibration curve. Slope, intercept and correlation coefficient of the calibration curves (peak area versus concentration) were determined to ensure linearity of the analytical method.

2.8.5. *Accuracy study and recovery*

Accuracy of the proposed method was confirmed by placebo spiking method, which was carried out by spiking a matrix of (lactose, Avicel, polyvinylpyrrolidone (pvp), starch and magnesium stearate) with metformin and gliclazide separately at 3 different levels 80%, 100% and 120%. Triplicate determinations of these 3 levels have been recorded to obtain the mean and % RSD.

2.8.6. *Method sensitivity, LOD and LOQ*

LOD and LOQ for metformin and gliclazide were calculated from the linear regression equation based on standard deviation of the intercept and the slope using the formula.

$$LOD = 3.3\ Q/S \text{ and } LOQ = 10\ Q/S$$

where Q: the standard deviation of the intercept, S: slope of the calibration curve.

2.8.7. *Robustness*

Deliberate minute variations in the chromatographic conditions such as flow rate, mobile phase composition and pH of the buffer component have been made. These variations were also evaluated for resolution between metformin and gliclazide peaks, number of theoretical plates and tailing factor.

3. Working solution stability

The stability of the gliclazide and metformin mixed solution (50 μg/ml each) was assessed after 24 h in autosampler, after 24 h at room temperature, 25 °C (light protected to minimize possible light degradation) and after a week in the fridge (2–8 °C).

4. Results and discussion

4.1. *Method development and optimisation*

A volatile, mass compatible ammonium formate buffer was selected in our study due to the following pKa values of both analytes – gliclazide and metformin. Ammonium formate has a pKa of 3.74. The low pH 3.5 selected for the separation falls within the buffering pH range (2.74–4.74) of ammonium formate and as close as possible to its pKa. At this low pH of 3.5, the ionization of the sulphonamide moiety on the gliclazide molecule (weak acid, pKa 5.8) [18] is suppressed. Metformin is a small polar molecule (pKa 2.8 and 11.5, log p Octanol: Water,-2.6) [18].

In all tried C18 columns, metformin was too polar to be retained with gliclazide on the same column with isocratic mode using different mobile phases mentioned earlier. Early trials of reducing the organic proportion in the mobile phase failed to retain metformin on any of the C18 columns used while a significant delay of a wide asymmetric peak of gliclazide has been noticed. The same findings have been reported by others [27] where metformin tends to be eluted

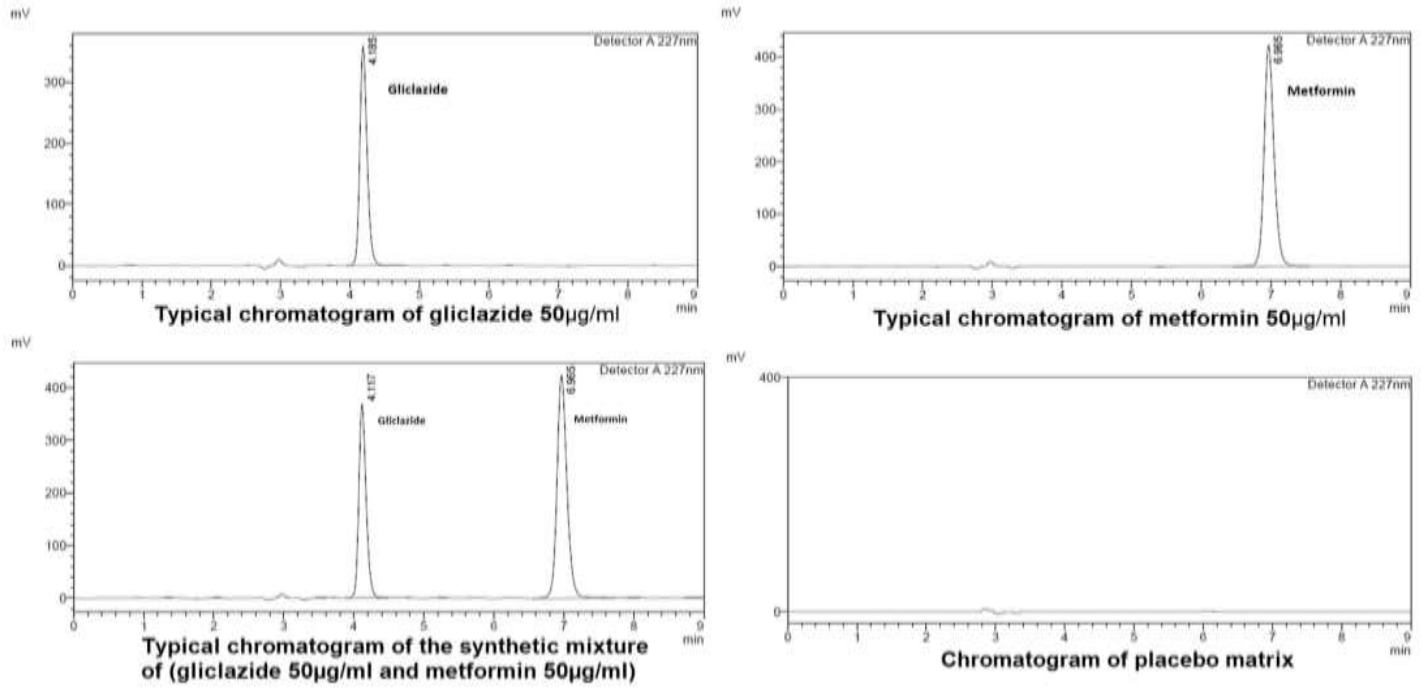

Fig. 1 – Chromatograms of placebo matrix, metformin, gliclazide and typical chromatogram of gliclazide and metformin mixture.

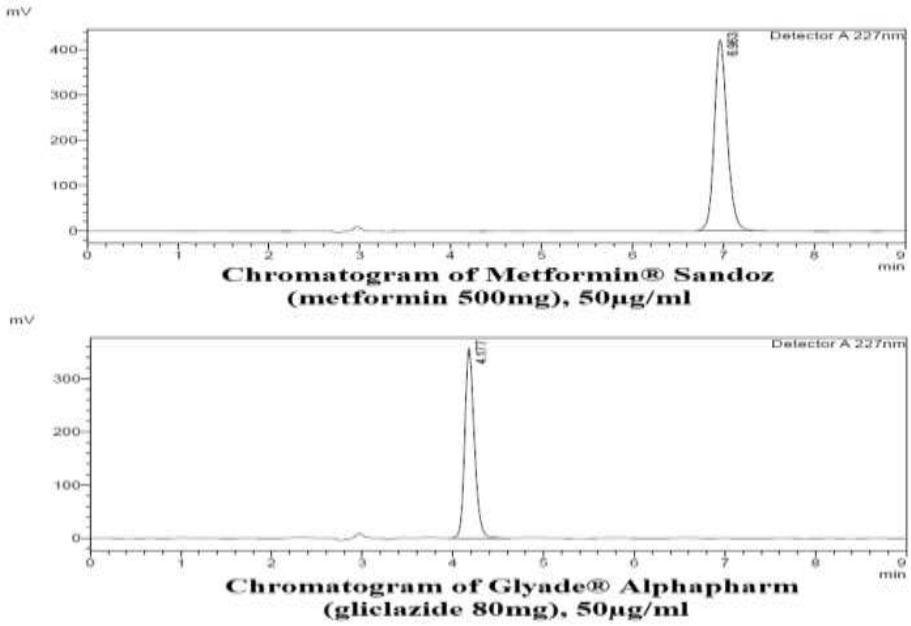

Fig. 2 – Chromatograms of Metformin® Sandoz (metformin 500 mg tablets) and Glyade® Alphapharm (gliclazide 80 mg tablets) both made to an injectable final dilution of 50 µg/ml.

rapidly from conventional C18 columns along with the dead volume of the column. Unretained metformin peak has been explained in another research where the polar metformin was not able to interact with lipophilic chains of the C18 stationary phase [21] and the difficulty in retaining metformin on conventional C18 columns or even with more polar phenyl columns has been reported in another investigation [28]. The authors of this research also commented on the use of other techniques such as cation exchange columns as a way of solving the unretained metformin problem. Additionally, the method is tedious, and the time consumed is a further limiting factor [28].

Ion pairing [20,21,27,28] and micellar liquid chromatography [22] are alternative techniques to conventional reversed phase chromatography that have been used to retain polar analytes such as metformin either alone or among other therapeutic agents. The time consumed in the preparation stage, mobile phase complexity and in some cases long chromatographic runs [20] are the major disadvantages of these techniques.

Table 2 – Recovery results for gliclazide and metformin (acceptance limit recovery % = 98–102%).

Gliclazide recovery results				
Sample name	Theoretical (claimed) concentration in µg/ml	The concentration found in µg/ml	Recovery %	Statistical data
S1 80%	42	41.76	99.42	Mean = 99.85
S2 80%		42.17	100.41	Standard deviation = 0.51
S3 80%		41.88	99.73	RSD% = 0.507%
S1 100%	54.6	54.89	100.54	Mean = 100.4
S2 100%		54.57	99.95	Standard deviation = 0.4
S3 100%		54.99	100.72	RSD% = 0.401%
S1 120%	63	62.99	99.99	Mean = 99.7
S2 120%		62.48	99.17	Standard deviation = 0.46
S3 120%		62.96	99.94	RSD% = 0.461%
Metformin recovery results				
S1 80%	43	42.65	99.20	Mean = 98.98
S2 80%		42.36	98.51	Standard deviation = 0.41
S3 80%		42.67	99.23	RSD% = 0.412%
S1 100%	50.2	50.96	101.52	Mean = 101.19
S2 100%		50.93	101.45	Standard deviation = 0.52
S3 100%		50.50	100.59	RSD% = 0.512%
S1 120%	60.2	60.66	100.77	Mean = 100.55
S2 120%		60.77	100.94	Standard deviation = 0.54
S3 120%		60.16	99.93	RSD% = 0.538%

The use of cyano columns, a more polar stationary phase than C18 columns, was also tested during the development phase of the proposed analytical method to investigate not only the interaction on a different stationary phase but also the chromatographic separation as well as the resolution between metformin and gliclazide peaks. The elution pattern on CN columns tried was opposite to what was noticed on all attempted C18 columns, where retention time of metformin was delayed on the chromatogram while gliclazide was eluted first using 20 mM ammonium formate buffer (pH 3.5) and acetonitrile. The observed retention times for both gliclazide and metformin were too close to be separated on Alltima HP CN (150 mm × 4.6 mm x 3μ) in the same chromatographic run, however the separation of either metformin alone or gliclazide alone on such a column could be optimized, so the decision was made to use a longer column yet the same stationary phase (cyano) in our study to have both eluents on the same chromatographic run.

In the present study, the use of Alltima CN (250 mm × 4.6 mm x 5μ) showed good retention of both metformin and gliclazide and the method was optimized via trying different mobile phase ratios. Eventually, a 20 mM ammonium formate buffer (pH 3.5) and acetonitrile at ratio of (45:55, v/v) gave the best chromatographic results and metformin was retained at 6.9 min while gliclazide was retained at 4.1 min on an isocratic mode without the need for ion pairing, micellar chromatography or even gradient elution with good sensitivity compared to others [12,23,24].

4.2. *System suitability*

The obtained results of 6 replicate injections showed that the parameters tested were within the acceptable range. Gliclazide and metformin were repeatedly retained and well separated at 4.1 min and 6.9 min expressing excellent resolution between both peaks with RSD% of the recorded retention times <0.3 to

Table 3 – Robustness results for both metformin and gliclazide (acceptance limit RSD% < 2).

Condition	Retention time		Tailing factor		Theoretical plates		Resolution
	glic	metf	glic	metf	glic	met	
Flow rate 0.9 ml/min	4.579	7.666	1.220	1.152	7436	12,148	12.583
Buffer (pH 3.5): Acetonitrile	4.582	7.663	1.213	1.154	7404	12,096	12.534
45:55, v/v	4.580	7.661	1.215	1.155	7435	12,085	12.544
mean	4.58	7.66	1.216	1.154	7425	12,109.67	12.55
Standard deviation	0.002	0.003	0.004	0.002	18.19	33.65	0.026
RSD%	0.033%	0.033%	0.297%	0.132%	0.245%	0.278%	0.206%
Flow rate 1 ml/min	4.151	6.911	1.228	1.160	6744	11,284	11.934
Buffer (pH 3.5): Acetonitrile	4.151	6.910	1.230	1.156	6743	11,264	11.927
45:55, v/v	4.151	6.909	1.229	1.157	6763	11,238	11.925
mean	4.151	6.91	1.229	1.158	6750	11,262	11.93
Standard deviation	0	0.001	0.001	0.002	11.269	23.065	0.0047
RSD%	0.000%	0.014%	0.081%	0.180%	0.167%	0.205%	0.040%
Flow rate 1.1 ml/min	3.761	6.283	1.245	1.169	6135	10,491	11.532
Buffer (pH 3.5): Acetonitrile	3.759	6.283	1.249	1.169	6146	10,514	11.554
45:55, v/v	3.758	6.285	1.248	1.167	6129	10,512	11.560
mean	3.759	6.284	1.247	1.168	6136.7	10,505.67	11.55
Standard deviation	0.002	0.001	0.002	0.001	8.621	12.741	0.015
RSD%	0.041%	0.018%	0.167%	0.099%	0.140%	0.121%	0.128%
Flow rate 1 ml/min	4.335	6.577	1.217	1.165	6882	10,838	9.712
Buffer (pH 3.5): Acetonitrile	4.335	6.577	1.215	1.164	6874	10,827	9.706
47.5:52.5, v/v	4.337	6.577	1.215	1.164	6893	10,821	9.702
mean	4.336	6.577	1.216	1.164	6883	10,828.67	9.707
Standard deviation	0.001	1.09	0.001	0.0006	9.54	8.62	0.005
RSD%	0.027%	0.000%	0.095%	0.050%	0.139%	0.080%	0.052%
Flow rate 1 ml/min	3.945	7.302	1.253	1.152	6478	11,729	14.418
Buffer (pH 3.5): Acetonitrile	3.949	7.297	1.254	1.152	6520	11,724	14.393
42.5:57.5, v/v	3.951	7.3	1.252	1.154	6525	11,721	14.394
mean	3.948	7.3	1.253	1.153	6507.67	11,724.67	14.4
Standard deviation	0.003	0.003	0.001	0.001	25.81	4.041	0.014
RSD%	0.077%	0.034%	0.080%	0.100%	0.397%	0.034%	0.098%
Flow rate 1 ml/min	4.088	6.964	1.231	1.156	6881	11,544	12.602
Buffer (pH 3.4): Acetonitrile	4.084	6.962	1.232	1.157	6885	11,560	12.626
45:55, v/v	4.083	6.96	1.221	1.158	6911	11,588	12.648
mean	4.085	6.962	1.228	1.157	6892.3	11,564	12.625
Standard deviation	0.003	0.002	0.006	0.001	16.289	22.271	0.023
RSD%	0.065%	0.029%	0.495%	0.086%	0.236%	0.193%	0.182%
Flow rate 1 ml/min	4.186	6.96	1.215	1.174	6834	11,147	11.898
Buffer (pH 3.6): Acetonitrile	4.189	6.959	1.215	1.173	6850	11,151	11.886
45:55, v/v	4.186	6.964	1.215	1.175	6875	11,128	11.92
mean	4.187	6.961	1.215	1.174	6853	11,142	11.901
Standard deviation	0.002	0.003	0	0.001	20.663	12.288	0.017
RSD%	0.041%	0.038%	0.000%	0.085%	0.302%	0.110%	0.145%

indicate good repeatability of replicate injections on the integral HPLC system used, the tailing factor for both gliclazide and metformin peaks never exceeded 1.25 in all peaks indicating good peak symmetry (acceptance limit is < 2) and the number of theoretical plates were always >2000 in all chromatographic runs to ensure good column efficacy throughout the developed separation process. Results are presented in Table 1.

4.3. Precision

The peak areas obtained following injecting 6 independent combined gliclazide and metformin samples were repeatable and precise over 3 consecutive days. The results for both intra-day and inter-day determinations ensure the high precision and repeatability of the designed method where, all data were expressed in RSD% and never exceeded 0.54% (acceptance limit RSD% <2). Results for intra and inter-day precision are given in Table 1.

4.4. Specificity

The analytical method was able to detect and assess metformin and gliclazide in the presence of placebo matrix of common tablet excipients. The representative chromatogram of, placebo, metformin standard, gliclazide standard and typical chromatogram of metformin and gliclazide mixture are shown in Fig. 1. The specificity of the method has been confirmed where the optimized conditions were applied to detect gliclazide and metformin (from manufacturer's excipients) in Glyade® Alphapharm tablets and Metformin® Sandoz tablets respectively, representative chromatograms of gliclazide peak in Glyade® Alphapharm tablets and metformin peak in Metformin® Sandoz tablets are shown in Fig. 2.

4.5. Linearity

The analytical calibration curve constructed for both gliclazide and metformin were linear in the specified ranges, indicated by the closeness of the correlation coefficient R^2 to 1 ($R^2 = 0.9999$). The linear regression equation for gliclazide is ($Y = 45392x + 27194$, $R^2 = 0.9999$) and the linear regression equation for metformin is ($Y = 99511x + 39966$, $R^2 = 0.9999$).

4.6. Recovery

Accuracy of the proposed analytical method was evaluated by determining the added analytes in the placebo matrix in triplicates at 3 different levels (80%, 100% and 120%) and expressed in terms of % recovery of metformin and gliclazide from the spiked matrix. The closeness of the values of found analytes compared to the claimed theoretical concentrations at different levels proved the trueness/accuracy of the proposed method where, metformin and gliclazide >99% recovered from the spiked excipients. Results for gliclazide and metformin recoveries are shown in Table 2.

4.7. LOD and LOQ

The calculated LOD and LOQ were 0.97 μg/ml, 2.95 μg/ml for gliclazide and 0.8 μg/ml, 2.45 μg/ml for metformin. The method sensitivity has been checked practically where experimental LODs were 0.8 μg/ml for both gliclazide and metformin and the experimental LOQs for both agents were 2.4 μg/ml.

4.8. Robustness

No significant changes detected upon applying small variations to the chromatographic conditions ensuring that the method is robust to small deliberate changes applied in terms of the flow rate, pH of the buffer used or different mobile phase ratios. In all cases gliclazide and metformin peaks were symmetric (tailing factor <2) and were well separated (resolution >2) and the RSD% of gliclazide and metformin retention times were <0.1 ensuring the robustness of the proposed analytical method to small changes. Results for robustness are presented in Table 3.

5. Solution stability

Gliclazide/metformin sample solution was stable for 24 h in both autosampler and 25 °C (room temperature) and after 1 week in fridge when maintained at 2–8 °C. The stability results have been assessed for the percentage difference from zero-time injections, where no decrease in the peak areas of either gliclazide or metformin have been detected in the mentioned conditions.

6. Conclusion

The presented validated method is rapid, economic, simple, accurate, sensitive, robust, specific and linear. It can be used for routine analysis of metformin and gliclazide either alone or in combination products.

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Chapter 4

Graphical abstract

Chapter 4: Advanced and multifaceted stability profiling of the first-line antidiabetic drugs metformin, gliclazide and glipizide under various controlled stress conditions

Gedawy A et al. 2020. "Advanced and multifaceted stability profiling of the first-line antidiabetic drugs metformin, gliclazide and glipizide under various controlled stress conditions"

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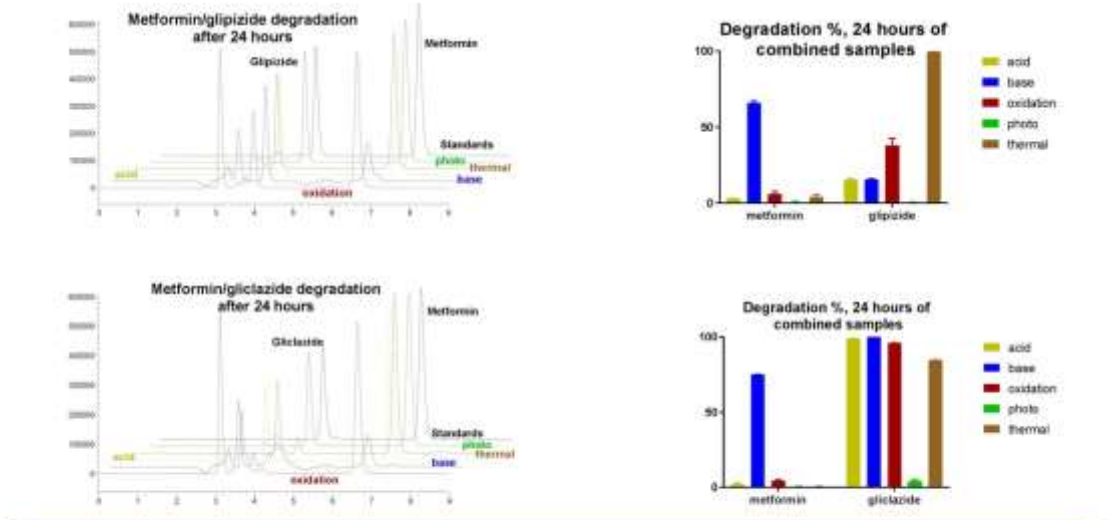

Degradation results of the binary mixtures of (metformin/glipizide) and (metformin/gliclazide) after 24 hours exposure to 1M HCl, 1M NaOH, sunlight, 85°C and 3% Hydrogen peroxide.

This chapter outlines the stability profiling of metformin and two sulfonylureas (gliclazide and glipizide) when subjected to various stress/degradation conditions. The study was conducted on individual analytes and on the binary mixtures of (metformin/gliclazide) and (metformin/glipizide). This stability indicating method was also applied for the analysis of marketed tablets of these antidiabetic agents after exposure to accelerated stability conditions.

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Original article

Advanced and multifaceted stability profiling of the first-line antidiabetic drugs metformin, gliclazide and glipizide under various controlled stress conditions

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ABSTRACT

The antidiabetic drugs metformin, gliclazide and glipizide have been widely used and studied in terms of pharmacological and antidiabetic effects, and their individual stability has been studied in the literature. However, the drugs' combined stability profiling remains poorly understood, and hence the aim of this study was to investigate the collective stability profiling of different combinations at various controlled conditions. Degradation assessments were carried out on metformin, glipizide and gliclazide by applying a stability-indicating HPLC method that was developed and validated in accordance with ICH guidelines. Glipizide, gliclazide, metformin and the binary mixtures (metformin/glipizide and metformin/gliclazide) were subjected to different forced degradation conditions and were detected at 227 nm by an isocratic separation on an Alltima CN column (250 mm × 4.6 mm × 5μ) utilizing a mobile phase that consists of 20 mM ammonium formate buffer (pH 3.5) and acetonitrile at a ratio of (45:55, v/v). The method is linear (R^2 = 0.9999) at the concentration range 2.5–150 μg/ml for metformin and 1.25–150 μg/ml for sulfonylureas respectively and offers a specific and sensitive tool for their determination in <10 min chromatographic run. All drug peaks were sharp and well separated. Stress degradation revealed that metformin has a remarkable sensitivity to alkaline stress, glipizide was more sensitive to thermal degradation while gliclazide exhibited almost full degradation in acidic, alkaline and oxidative stress conditions.

1. Introduction

Glipizide and gliclazide are second-generation sulfonylureas (Tommasini,1975; Palmer and Brogden,1993). Sulfonylureas are the oldest oral therapies for type 2 diabetic patients and were mainstay treatment since 1956 (Thulé and Umpierrez,2014).

Metformin was discovered in the 1950s for treatment of diabetic patients (Bell and Hadden,1997). Metformin is the recommended first-line therapy for type 2 diabetic patients around the world, according to international guidelines (Rena et al., 2013).

In our previous work, we have developed and validated a new HPLC method for simultaneous determination of metformin and gliclazide (Gedawy et al., 2019). In the current paper we are investigating the suitability of this new method to serve as a stability indicating tool for the analysis of gliclazide, metformin and another sulfonylurea (glipizide) when subjected to degradative stress conditions and applying the method on commercial samples of these pharmaceutical products post accelerated stability assessment. Literature survey reveals stress degradation studies that have been performed on metformin alone (Cristina Stenger et al., 2012), glipizide alone (Gupta and Bansal, 2011; Bansal et al., 2008a), gliclazide alone (Doomkaew et al., 2015; Bansal et al., 2007), gliclazide and glipizide together (Gumieniczek et al., 2014) or metformin and other agents (Sri Lakshmi et al., 2015; Vaingankar and Amin, 2016; Gite and Patravale, 2015). In one HPLC degradation study where both glipizide and metformin were studied, the authors reported glipizide degradation under various conditions while metformin did not show any degradation at the hydrolytic conditions tested (Sri Lakshmi et al., 2015), although degradation of metformin has been reported by others (Vaingankar and Amin,

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2016; Ramesh and Habibuddin, 2014). Gliclazide degradation has been reported in two papers (Gumieniczek et al., 2014; Bansal et al., 2007), though one paper stated that gliclazide could withstand alkaline degradation (Doomkaew et al., 2015). The aim of the current study was to carry out a forced degradation study to investigate the degradation behavior of the binary mixtures (metformin/gliclazide and metformin/glipizide) versus control (individual analytes) when subjected to different forms of forced degradation. This method is to our knowledge, the first stability-indicating HPLC method to compare the degradation results of metformin with two different sulfonylureas and can serve as an accurate, selective and cost-effective analytical tool for simultaneous analysis of fixed dose combinations of metformin and sulfonylureas.

2. Experimental

2.1. Materials and reagents

Metformin hydrochloride 97%, gliclazide greater than 98%, glipizide and analytical reagent grade ammonium formate were procured from Sigma-Aldrich (St. Louis, USA). Metformin® (metformin 500 mg tablets, Sandoz, Sydney, Australia), Glyade® (gliclazide 80 mg tablets, Alphapharm, Brisbane, Australia) and Melizide® (glipizide 5 mg tablets, Alphapharm, Brisbane, Australia) were obtained from a local pharmacy. Formic acid (analytical grade) was obtained from Ajax Fine Chemicals Pty Ltd (Melbourne, Australia). Acetonitrile (HPLC grade) was purchased from Thermo Fisher Scientific (Melbourne, Australia). Distilled water for buffer and sample preparations have been obtained from a Milli-Q ultra-pure water system (Millipore, Australia).

2.2. Instrumentation

Table 1 summarizes The HPLC system used and the chromatographic conditions of the entire study. Labsolutions version 5.82 was used for data acquisition and chromatographic integration.

2.3. Preparation of standard stock solutions (glipizide and metformin) for method validation

Glipizide 25 mg/100 ml (Flask A) and Metformin 25 mg/100 ml (Flask B) were prepared as described in our previous work (Gedawy et al., 2019).

2.4. Preparation of working solution (glipizide and metformin mixture) for method validation

Synthetic mixture of glipizide and metformin (50 μg/ml) each was prepared by transferring 2 ml from Flask A and 2 ml from Flask B into a 10 ml flask and final volume made up with mobile phase mixture (Gedawy et al., 2019).

Table 1
HPLC instrumentation and the chromatographic conditions of the optimized method.

Instrument	Shimadzu HPLC, Japan equipped with a LC-20AT pump with inline degasser), (SPD-20A $_{5R}$ UV detector) and (SIL-20AC $_{HT}$ Autosampler).
Column	Alltima CN (250 mm × 4.6 mm × 5μ), air-conditioned laboratory (25 °C)
Detector	UV
Wavelength	227 nm
Mobile phase	20 mM ammonium formate, pH 3.5 and acetonitrile, (45 :55, v/v)
Run time	10 min
Flow rate	1 ml/min
Injection volume	20 μl

2.5. Forced degradation studies

Forced degradation studies were conducted on control samples containing glipizide 1 mg/ml (Flask C), gliclazide 1 mg/ml (Flask D), metformin 1 mg/ml (Flask E) and the combined samples of glipizide/metformin mixture1mg/ml each (Flask F) and gliclazide/metformin mixture1mg/ml each (Flask G). An aliquot of 10 ml from flask C, D, E, F or G were transferred into a 20 ml volumetric flask and the final volume was made up to 20 ml using 1 M HCl (in a thermostat water bath 45 °C, 24 h) for acid degradation, 1 M NaOH (in a thermostat water bath 45 °C, 24 h) for alkaline degradation, 3% H_2O_2 (in a thermostat water bath 45 °C, 24 h) for oxidative stress and finally with the mobile phase mixture for photolytic (total of 24 h in sunlight) and thermal degradation studies (in a thermostat oven 80 °C, 24 h). These stressed samples were finally diluted to make 50 μg/ml control individual drugs or combined mixtures 50 μg/ml each. Note that oxidative and thermally stressed samples were cooled only, while acid and alkaline stressed samples were cooled and neutralized with 1 M NaOH and 1 M HCl respectively before the final dilution step.

Individually stressed glipizide, gliclazide and metformin samples (control) as well as the combined synthetic mixtures of (metformin/glipizide) and (metformin/gliclazide) were analysed in triplicates immediately after treatment and after 24 h. Note that blank acid, blank alkali and blank oxidation samples were injected to visualize and eliminate any possible unwanted new solvent peaks from the obtained results.

% Drug degradation was calculated using the formula:

$$\%\textbf{\textit{Drug degradation}} = \frac{\text{Area of unstressed sample} - \text{Area of stressed sample}}{\text{Area of unstressed sample}} \text{X}100$$

2.6. Stability testing of glipizide, gliclazide and metformin tablets

Five blister strips of Melizide® (glipizide 5 mg tablets), Glyade® (gliclazide 80 mg tablets), and Metformin® (metformin 500 mg tablets) were kept at 4 °C for four weeks while five other blister strips of the same batch of each product were placed in an accelerated stability chamber at 40 °C and 75% relative humidity for the same period. After four weeks, accelerated stability samples as well as refrigerated samples were left on the bench for one hour to reach ambient temperature before they got processed for content uniformity. 20 tablets of Melizide® were weighed and crushed. 204.8 mg powder equivalent to one Melizide® tablet (5 mg glipizide) was placed in a 50 ml volumetric flask. 5mls of distilled water was then added, sonicated for 10 min and final volume was made up to the mark using the mobile phase mixture. An aliquot of 10 ml of this flask was transferred to 20 ml volumetric flask and final volume was made up to the mark using the mobile phase mixture to produce 50 μg/ml glipizide flask. 50 μg/ml metformin and 50 μg/ml gliclazide flasks from Metformin® Sandoz 500 mg tablet (average weight, 583.35 mg) and Glyade® Alphapharm 80 mg tablet (average weight, 157.3 mg) tablets were prepared as mentioned in our earlier work (Gedawy, Al-Salami and Dass,2019). Metformin, glipizide and gliclazide content was determined as follows:

$$\%\textbf{\textit{Drug assay}} = \frac{\textbf{At}}{\textbf{As}} \text{X} \frac{\textbf{Cs}}{\textbf{Ct}} \text{X} \frac{\text{Average tablet weight (mg)}}{\text{Label drug claim (mg)}} \text{X} \frac{\textbf{P}}{100} \text{X}100$$

where, **At** is the peak area of test tablet, **As** is the peak area of reference standard.

Cs is the concentration of standard, **Ct** is the concentration of test tablet and **P** is the percentage purity of reference standards used.

2.7. Analytical method validation on metformin/glipizide mixture.

The stability indicating method for simultaneous determination of glipizide and metformin has been validated as per ICH guidelines Q2R1 (ICH, 2005) for evaluating linearity, accuracy, precision, specificity, system suitability, limit of detection (LOD), limit of quantitation (LOQ) and robustness.

2.7.1. System suitability and precision intra-day precision (repeatability) and inter-day precision (intermediate precision)

System suitability parameters with respect to number of theoretical plates, repeatability, tailing factor and resolution between glipizide and metformin peaks were assessed by injecting a blank mobile phase followed by six replicates of glipizide/metformin mixture at a concentration of 50 μg/ml each. Six independent combined samples of glipizide and metformin (50 μg/ml each) were injected on the same day under same operating conditions for system and method precision study while inter-day precision was assessed by comparing the results of 6 independent determinations on three consecutive days.

2.7.2. Linearity and range

Flask A - the standard stock solution of glipizide was diluted in the concentration range (1.25–150 μg/ml). Triplicates of each concentration were analysed and plotted on a glipizide calibration curve. Metformin from (Flask B) was diluted in the concentration range of (2.5–150 μg/ml), and assayed in triplicates, then plotted on a metformin calibration curve. Intercept, slope and correlation coefficient of the calibration curves (peak area versus concentration) were determined to ensure linearity of the proposed analytical method.

2.7.3. Accuracy study and recovery

Accuracy of the proposed method was confirmed by spiking the synthetic mixture of potato starch, magnesium stearate, polyvinylpyrrolidone (PVP), microcrystalline cellulose and anhydrous lactose with glipizide and metformin separately at 3 different levels 80%, 100% and 120%. Triplicate determinations of these 3 levels were recorded to obtain the mean and % RSD.

2.7.4. Method sensitivity, limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ for glipizide and metformin were calculated from the linear regression equation based on the slope and standard deviation of the intercept using the formula

$$\text{LOD} = 3.3\,Q/S \quad \text{and} \quad \text{LOQ} = 10\,Q/S$$

where Q is the standard deviation of the intercept, and S is the slope of the calibration curve.

2.7.5. Robustness

Intended small variations in the chromatographic conditions such as mobile phase composition and flow rate have been made. These variations were also evaluated for tailing factor, number of theoretical plates and resolution between glipizide and metformin peaks.

3. Results and discussion

3.1. Forced degradation results

In the present study, it was shown that the optimized HPLC method could serve as a stability indicating procedure where it is able to detect and quantify metformin, gliclazide and glipizide in the presence of their degradation peaks (Figs. 1, 2). Immediately after acidic treatment, metformin was more stable than sulfonylureas (with<1% degradation, order of stability is metformin > glipizide > gliclazide) (Fig. 1a). On the other hand, gliclazide per se was more labile than metformin and glipizide to both acidic and basic degradation. Gliclazide also has the least stability towards acid in metformin/gliclazide sample (Fig. 1b) verifying the reported sensitivity of sulfonylureas and heterocyclic rings towards acidic and alkaline hydrolysis (Bansal et al.,2008b). Although its relative stability towards alkaline hydrolysis in control sample (Fig. 1a), glipizide has initially lower stability towards alkali when combined with metformin (Fig. 1c). Initial oxidative stress revealed that metformin is more stable than sulfonylureas in control as well as combined synthetic mixtures (Fig. 1).

Photodegradation results revealed that metformin is the most resistant to light degradation of all analytes over 24 h while gliclazide is the most sensitive to light in control samples (Fig. 2a) and in combination sample (Fig. 2h). Glipizide on the other hand, showed almost full degradation to thermal conditions after 24 h (more than 99% degradation of initial glipizide concentration) (Fig. 2a) under the specified wet thermal conditions for both control and glipizide/metformin combined samples and almost full conversion into a degradation product that appeared on the chromatogram at a retention time of 3.63 min while the original glipizide peak retained at 4.02 min (Fig. 2c, f). These results confirm the high susceptibility of glipizide to heat and humidity reported by Gupta and Bansal (Gupta and Bansal,2011). At the end of 24 h, gliclazide exhibited more than 84% degradation under the same wet thermal conditions alone (Fig. 2a) and when combined with metformin (Fig. 2h), while metformin has negligible thermal degradation outcomes.

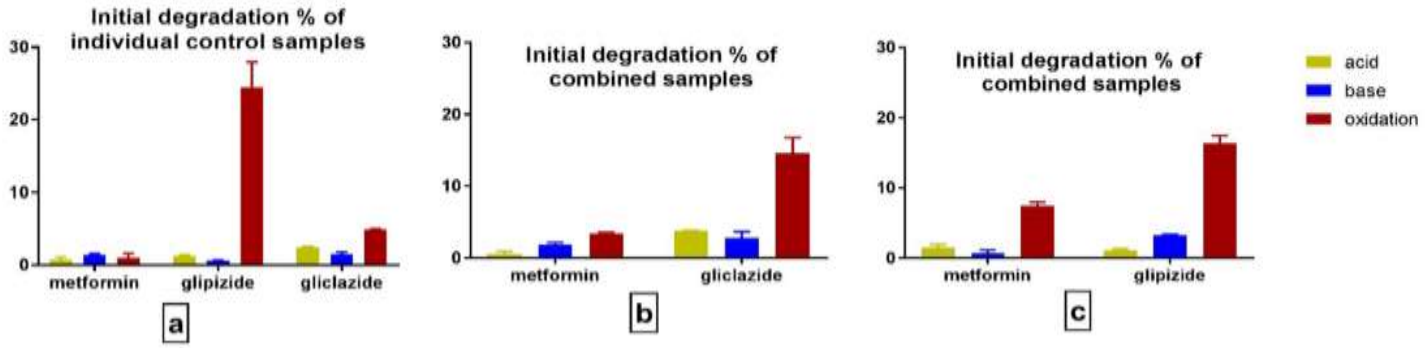

Fig. 1. Initial degradation results, immediately after treatment with acid, base and 3% H_2O_2 of **a**, control samples of metformin (50 μg/ml)(n = 3), glipizide (50 μg/ml)(n = 3), gliclazide (50 μg/ml)(n = 3) versus **b**, combined metformin/gliclazide sample (n = 3) and **c**, combined metformin /glipizide sample (n = 3).

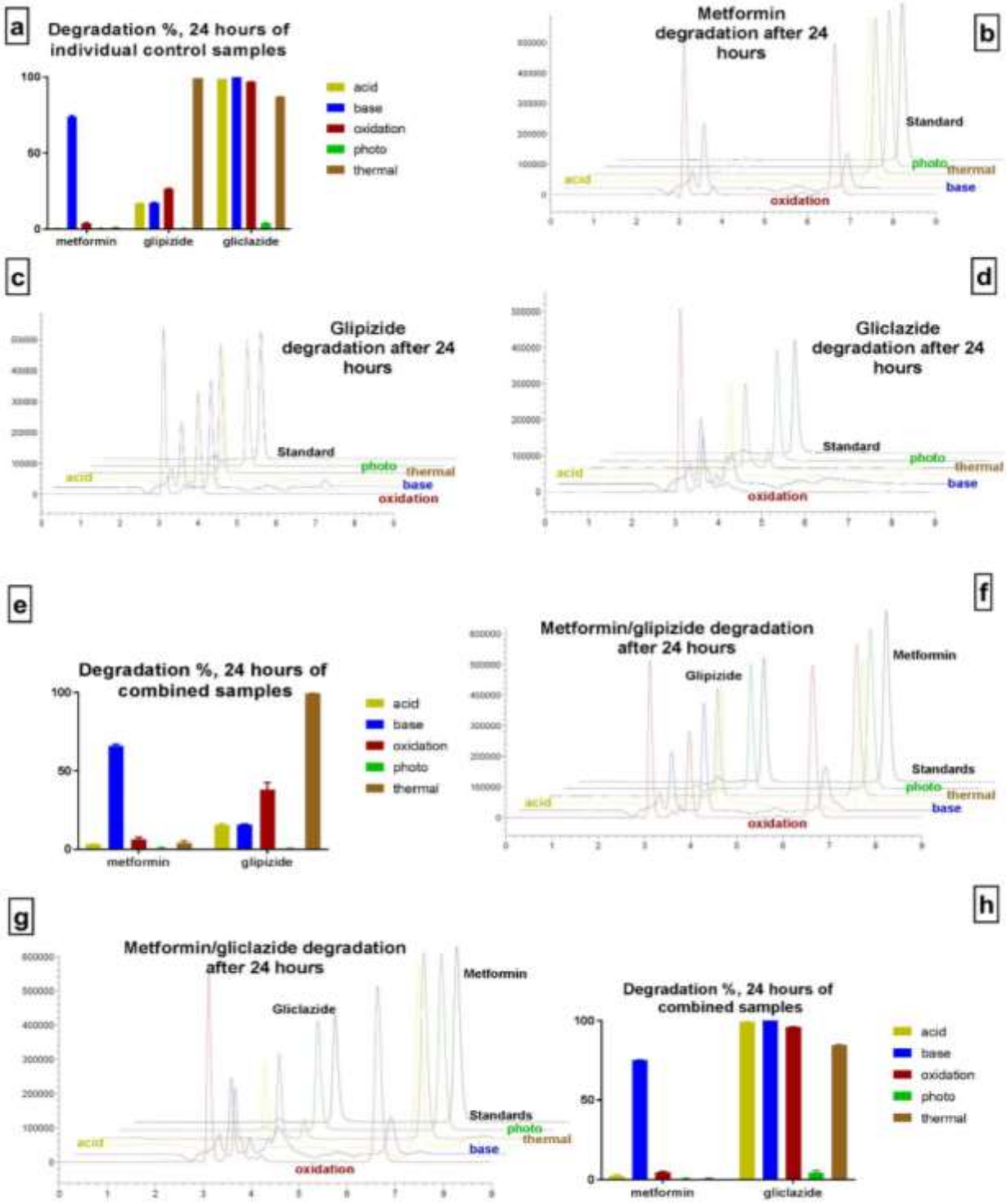

Fig. 2. Degradation results after 24 h exposure to acid, base, 3% H_2O_2, Sunlight and 85 °C heat of **a**, control samples of metformin (50 μg/ml)(n = 3), glipizide (50 μg/ml)(n = 3), gliclazide (50 μg/ml)(n = 3) versus **e**, combined metformin/glipizide sample (n = 3) and **h**, combined metformin /gliclazide sample (n = 3).

Metformin also was more stable than the two sulfonylureas under the tested acidic conditions alone and in combination samples while gliclazide showed more than 98.5% alone and when combined with metformin in acid (Fig. 2a, h). Glipizide exhibited close acidic degradation results (16.86%) in control sample (Fig. 2a) and (15.37%) when combined with metformin after 24 h (Fig. 2e). Despite reported gliclazide stability to alkaline hydrolysis in one paper (Doomkaew et al., 2015), full degradation of gliclazide was noted in our experiments (Fig. 2a, h), where no gliclazide peak was detected after 24 h in either gliclazide control (Fig. 2d) or gliclazide/metformin combined sample (Fig. 2g) which confirms previous gliclazide alkaline degradation results (Gumieniczek et al.,2014; Bansal et al., 2007; Bansal et al., 2008b). Glipizide was the most resistant to basic degradative stress and recorded (17.2%) degradation in control sample (Fig. 2a) compared to (15.69%) when combined with metformin (Fig. 2e). These results

Table 2
Chromatographic system suitability and precision results.

System suitability parameters (acceptance limit RSD % <2)							
	Retention time		Tailing factor		Number of theoretical plates		Resolution between both peaks
	glipizide	metformin	glipizide	metformin	glipizide	metformin	
1	4.015	6.967	1.193	1.156	6342	11,245	12.712
2	4.015	6.966	1.194	1.163	6340	11,213	12.699
3	4.012	6.965	1.194	1.162	6359	11,252	12.728
4	4.014	6.966	1.195	1.163	6358	11,244	12.721
5	4.014	6.966	1.197	1.165	6325	11,209	12.696
6	4.015	6.966	1.192	1.167	6360	11,218	12.705
Mean	4.0141	6.966	1.194	1.163	6347.33	11230.17	12.71
Standard deviation	0.0012	0.0006	0.0017	0.0037	14.08	18.86	0.0126
RSD%	0.029%	0.009%	0.144%	0.320%	0.222%	0.168%	0.099%
Precision results as peak area of different determinations on 3 different days (metformin 50 μg/ml, glipizide 50 μg/ml) (n = 6), acceptance limit RSD% <2).							
	Day 1		Day 2		Day 3		
	glipizide	metformin	glipizide	metformin	glipizide	metformin	
1	3,357,977	4,187,679	3,305,867	4,237,157	3,216,249	4,587,130	
2	3,375,004	4,216,894	3,322,221	4,238,781	3,218,643	4,569,885	
3	3,343,240	4,193,075	3,322,101	4,233,311	3,223,746	4,565,111	
4	3,345,503	4,193,694	3,300,586	4,276,047	3,229,361	4,568,475	
5	3,365,396	4,209,394	3,318,554	4,239,468	3,216,469	4,566,134	
6	3,373,302	4,223,693	3,308,166	4,285,244	3,222,432	4,577,338	
Mean	3360070.3	4204071.5	3312915.8	4,251,668	3,221,150	4572345.5	
Standard deviation	13611.05	14663.4	9241.36	22733.88	5060.04	8431.63	
RSD%	0.405%	0.349%	0.279%	0.535%	0.157%	0.184%	

confirm the stability of glipizide over gliclazide under the acidic and basic conditions reported by Gumieniczek et al (Gumieniczek et al., 2014).

Literature survey revealed that glipizide degradation rate in acidic conditions was equivalent to that in alkaline medium (Bansal et al., 2008a; Gumieniczek et al., 2014). Our results confirm these findings, where after 24 h of stressing glipizide control sample to the specified acidic and basic conditions, almost the same degradation percentage was obtained, namely 16.86% (in acidic) and 17.2% (in alkaline) (Fig. 2a). The same pattern of glipizide degradation was noticed in the combined glipizide/metformin mixture, 15.37% (in acidic) compared to 15.69% (in alkaline) (Fig. 2e). Vulnerability of glipizide to hydrolytic degradation is attributed to the presence of amide and the sulfonylurea moiety in its chemical structure (Bansal et al., 2008a). Glipizide and gliclazide degradation has been previously reported to follow the first order kinetics (Gumieniczek et al., 2014). In our study, around 74% degradation of metformin was observed in the control sample (Fig. 2a) and more than 66% degradation in combined samples (Fig. 2e, h). The clear susceptibility of metformin towards alkaline stress than acid confirm previous results (Gite and Patravale, 2015; Vaingankar and Amin, 2016; Cristina Stenger et al., 2012; Hamdan et al., 2010) although other researchers reported its stability to all hydrolytic conditions (Sri Lakshmi et al., 2015).

Sulfonylureas showed higher sensitivity towards oxidative stress than metformin where more than 96% degradation of gliclazide was recorded in both control and gliclazide/metformin samples (Fig. 2a, h). Glipizide on the other hand, recorded 26.28% degradation in control sample (Fig. 2a), 38% degradation in glipizide/metformin sample (Fig. 2e) under same conditions, while metformin had minimal oxidative degradation.

3.2. Stability testing and tablet assay results

The stability indicating HPLC method was successfully used to assay three different commercial metformin, glipizide and gliclazide products in stability chamber (40 °C, 75% relative humidity) compared to control fridge samples. Metformin® (metformin 500 mg tablets), Melizide® (glipizide 5 mg tablets) and Glyade® (gliclazide 80 mg tablets) exhibited 100% content uniformity at 4 °C while stability chamber samples of Metformin®, Melizide® and Glyade® assayed 100%, 99.94% and 99.96% respectively after 4 weeks.

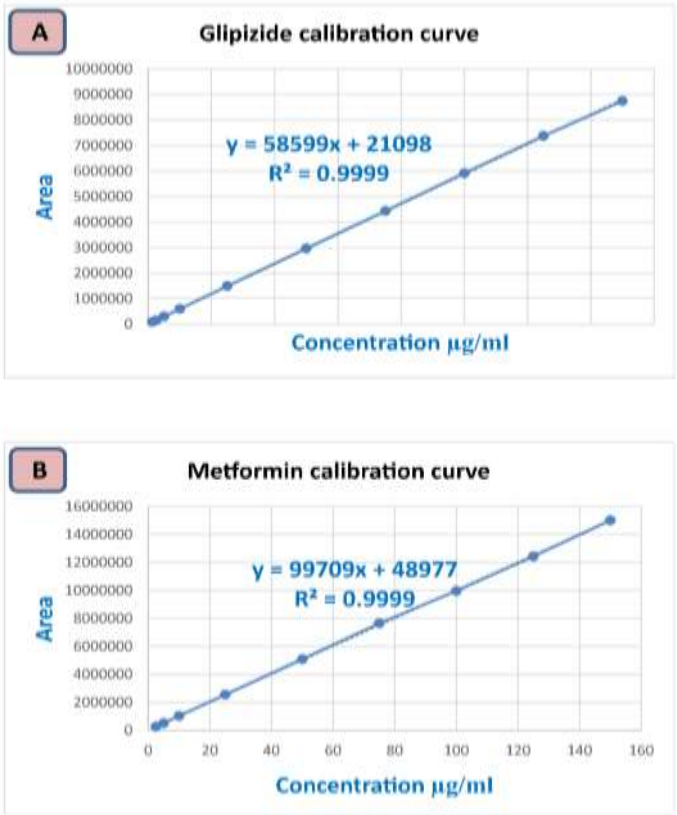

Fig. 3. Linearity curves of glipizide (A) and (B) metformin.

Table 3
Recovery results for glipizide and metformin.

Sample name	Theoretical (claimed) concentration in μg/ml	The concentration found in μg/ml	Recovery %	Statistical data
Glipizide recovery study (acceptance limit recovery % = 98–102%).				
gp1 80%	41	41.59	101.44	Mean = 100.5
gp2 80%		41.11	100.28	Standard deviation = 0.83
gp3 80%		40.93	99.83	RSD% = 0.826%
gp1 100%	50.2	49.4	98.4	Mean = 98.8
gp2 100%		49.75	99.11	Standard deviation = 0.38
gp3 100%		49.68	98.97	RSD% = 0.381%
gp1 120%	59.8	59.62	99.7	Mean = 99.4
gp2 120%		59.48	99.47	Standard deviation = 0.35
gp3 120%		59.21	99.02	RSD% = 0.35%
Metformin recovery study (acceptance limit recovery % = 98–102%).				
mt1 80%	42	42.48	101.15	Mean = 100.46
mt2 80%		42.07	100.17	Standard deviation = 0.597
mt3 80%		42.03	100.07	RSD% = 0.594%
mt1 100%	51	50.89	99.79	Mean = 99.6
mt2 100%		50.82	99.66	Standard deviation = 0.22
mt3 100%		50.68	99.37	RSD% = 0.216%
mt1 120%	60.4	60.43	100.05	Mean = 100.03
mt2 120%		60.57	100.29	Standard deviation = 0.28
mt3 120%		60.24	99.74	RSD% = 0.28%

3.3. System suitability and precision

Results of 6 replicate injections are presented in Table 2 and the parameters tested were within the acceptable limits. Metformin and glipizide peaks were sharp (tailing factor < 1.2) and well separated (resolution greater than 2) and repeatedly retained at 6.96 min for metformin and 4.01 min for glipizide in all injections. Peak areas resulted from injecting 6 independent combined metformin and glipizide samples were precise and repeatable over 3 days, where intra and inter-day determinations verified the repeatability and precision of the proposed method. All data were compliant with ICH requirements and expressed in RSD% (acceptance limit, RSD% <2). Results for intra and inter-day precision are given in Table 2.

3.4. Linearity

The analytical calibration curves constructed for both glipizide and metformin were linear in the specified ranges. The linear regression equation for glipizide was ($y = 58599 \times + 21098$, $R^2 = 0.9999$) Fig. 3a, and the linear regression equation for metformin was ($y = 99709 \times + 48977$, $R^2 = 0.9999$) Fig. 3b.

3.5. Recovery

98.8–100.5% glipizide recovered from the spiked excipients and 99.6–100.46% metformin recovery were recorded to prove the accuracy/trueness of the proposed analytical method.

Results for glipizide and metformin recovery are shown in Table 3.

3.6. Limit of detection (LOD) and limit of quantitation (LOQ)

The calculated LOD and LOQ were 0.796 μg/ml, 2.412 μg/ml for glipizide respectively and 1.069 μg/ml, 3.24 μg/ml for metformin respectively. Experimental results showed that the method was able to detect as low as 0.8 μg/ml of both glipizide and metformin and to quantify as low as 2.4 μg/ml of both too.

3.7. Robustness

No significant changes were noticed with small variations ensuring that the method used is robust to small intended changes in chromatographic conditions. In all cases, metformin and glipizide peaks were symmetric (tailing factor < 2) and were well separated (resolution greater than 2) and the RSD% of metformin and glipizide retention times were <0.2 ensuring the robustness of the proposed analytical method to small changes (data not shown).

4. Conclusions

The proposed stability indicating method presents a simple and rapid utility for simultaneous determination of sulfonylureas and metformin in bulk and in pharmaceutical preparations and can be used for routine quality control of single agents or metformin/-sulfonylurea combination tablets. The proposed method exhibited good accuracy and robustness with a linear response behavior. Due to the mass compatibility of the mobile phase used, the method can also be optimized for LC/MS application for future work. Forced degradation results confirmed the specificity and the ability of the method to detect and quantify sulfonylureas and metformin among their degradation peaks which enables the use of this method for stability studies of glipizide, gliclazide and metformin preparations.

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Chapter 5

Chapter 5: General discussion and future perspective

The journey of metformin in the last century has been quite eventful, from discovery of its blood glucose lowering potential in the 1930's [Slotta et al 1929] [Hesse et al 1929], rediscovery and repurposing in the 1940's as antimalarial and anti-influenza [Garcia 1950], then it's abandonment due to insulin discovery. Metformin was in fact 'rescued' in 1957 when its role in diabetes management and the detailed pharmacotherapeutics of this biguanide were published [Baily 2017]. Despite the concerns of lactic acidosis associated with some biguanides and withdrawal of buformin and phenformin from certain markets, metformin was able to survive, and it was listed in the essential medicines of World Health Organization (WHO) in 2011 [Baily 2017]. During this journey, very little of the cellular mechanisms of this unique biguanide were identified, and to date, the exact molecular mechanism(s) of metformin action is not fully understood. Further studies into this are warranted given its role in non-glycaemic effects in various pathologies such as (cancer, inflammation, neurodegenerative diseases and geroprotection).

Marketed metformin products still suffer from incomplete oral absorption and short half-life with overall limited oral bioavailability [Scheen 1996] [Graham et al 2011]. Microencapsulation is an innovative discipline that uses various polymeric materials to improve the oral bioavailability of pharmaceuticals, enhance their stability, modify their pharmacokinetic properties and allow for organ/tissue targeting, in the hope that better pharmacodynamics can be achieved. Masking unpleasant taste of many pharmaceuticals, sustaining, delaying, prolonging or controlling the release characteristics of various drugs are all privileges offered by microencapsulation. Yet, low drug loading, limited encapsulation efficiencies and sometimes the need of special equipment could be limitations of some encapsulation techniques [Jyothi et al 2010] [Jyothi et al 2012] [Chen et al 2019].

In this thesis, I developed novel silicon-based microcapsules by grafting polydimethylsiloxane (PDMS) with alginate to form stable self-emulsified pharmaceutical vehicle [Gedawy et al 2020b]. This new polymeric platform exhibited good electro-kinetic stability and showed non-Newtonian shear thinning rheological properties, a common behavior in pharmaceuticals. The new therapeutic platform was then used to encapsulate metformin by utilising vibrational jet nozzle ionotropic gelation technique to produce discrete spherical microcapsules [Gedawy et al 2020b].

These microcapsules showed good flow properties with a uniform particle size distribution. No chemical incompatibilities were identified between metformin and any of the polymers used to formulate these microcapsules. Significant improvements in metformin loading and the encapsulation efficiency were noticed in silicon-based

metformin microcapsules compared to control [Gedawy et al 2020b]. PDMS-grafted alginate platform showed good stability properties to encapsulate metformin and after four weeks, no change in the metformin contents of any of the siliconised microcapsules were identified.

The swelling properties of PDMS microcapsules in simulated gastric and intestinal conditions were dependent on the PDMS content, where microcapsules formulated with higher PDMS content underwent less swelling in these media compared to control [Gedawy et al 2020b]. The hydrophobic nature of this silicon polymer resisted the aqueous uptake from the tested media, where the swelling characteristics were chiefly attributed to the alginate content of the polymeric platform. The swelling behaviour of PDMS microcapsules in simulated intestinal fluid was much higher than that in acidic conditions, which is ideal for metformin microcapsules to withstand gastric conditions and undergo swelling and loosening of the polymeric backbone in simulated intestinal conditions, thus facilitating release of their drug payload [Gedawy et al 2020b].

Chromatographic detection and analysis of metformin are often problematic due to its small molecular size and polar nature, making it difficult to retain this molecule on many chromatographic columns. Some techniques such as micellar liquid chromatography [El-Wasseef 2012], ion pairing chromatography [Eva et al 2016], gradient elution [AbuRuz et al 2005] or even the use of special chromatographic columns [AbuRuz et al 2003] are often needed to detect and quantify metformin among other analytes. Most of these approaches are time-consuming and sophisticated, and the long chromatographic runs are a further limitation.

Sulfonylureas have been in use in diabetes management since 1956 due to their safety profiles [Thule et al 2014]. They are considered second line therapy for diabetes and sometimes first line if metformin is not tolerated. The combination of metformin and sulfonylureas is a common strategy suggested by many clinicians and diabetologists for tight control of the blood glucose level of diabetic patients [Blonde et al 2012].

In this thesis, I developed a new and simple analytical HPLC method for simultaneous isocratic analysis of metformin and two members of sulfonylureas (glipizide and gliclazide) [Gedawy et al 2019] [Gedawy et al 2020a]. Of note, this method was able to detect and quantify these therapeutics in less than 10 minutes either as individual analytes or when the basic, water-soluble metformin is combined with one of these acidic, water-insoluble sulfonylureas.

This new method was validated according to International Conference of Harmonisation (ICH) guidelines Q2 (R1) in terms of accuracy, linearity, specificity, system suitability, precision, robustness, limit of detection and limit of quantification. The method has high selectivity for the analysis of these antidiabetics in a linear response (R^2,0.9999) and

showed high accuracy (% Recovery, > 98%), excellent precision (RSD%, < 0.7%) and good robustness towards minor alterations in chromatographic conditions (RSD %, < 0.6%) and could detect as low as 0.8µg/ml of metformin, glipizide and gliclazide.

I applied the new method to test the stability of metformin, glipizide and gliclazide when subjected to various degradation conditions of sun light, strong acid (1M HCl), strong base (1M NaOH), high temperature (80^{o}C) and oxidation (3% H_2O_2). The method was able to detect and quantify these antidiabetic agents among their degradation peaks [Gedawy et al 2020a].

The chromatographic method was also applied for the simultaneous analysis of marketed tablets of metformin, glipizide and gliclazide in less than 10 minutes and it was used to test the stability of these marketed products after 4 weeks of accelerated stress conditions (40^{o}C, 70% relative humidity). The new analytical method can serve as a rapid analytical tool for detection and quantification of metformin, glipizide and gliclazide for research purposes or industrially in quality control laboratories for routine analysis of these antidiabetics as well as their stability testing either individually or as binary mixtures of (metformin/glipizide) and (metformin/gliclazide) [Gedawy et al 2020a].

Limitations

Despite the high production yield of microcapsules produced with laminar jet flow ionotropic gelation technique, metformin loading and the encapsulation efficiency of the produced PDMS microcapsules still needs to be improved to allow for meaningful *in vitro* and *in vivo* release studies of PDMS microcapsules. On the other hand, the new analytical technique developed in this thesis can be optimised for liquid chromatography combined with mass spectroscopy to allow for wider range of applications in the detection and analysis of biguanides and sulfonylureas in various biological samples.

Future perspective

New encapsulation conditions, alteration in drug to polymer ratio, other encapsulation techniques, different silicon polymers or combination of all these conditions might be needed to improve and optimise the drug loading and encapsulation efficiency in PDMS-customised platforms to allow for versatile pharmaceutical applications and the therapeutic delivery of various payloads.

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Appendix A: Relevant publication and conference poster

Relevant publication to the thesis but not forming a part of it

- **Gedawy A**, Martinez J, Al-Salami H, Dass CR. Oral insulin delivery: existing barriers and current counter-strategies. Journal of Pharmacy and Pharmacology. 2018;70(2):197-213.

Conference poster

- **Gedawy A**, Al-Salami H, Dass CR. Novel analytical method for simultaneous determination of the binary mixture of antidiabetic drugs, metformin and gliclazide.

  Poster presented at the 2018 Mark Liveris Symposium, Bentley campus, Curtin University, Western Australia.

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Review

Oral insulin delivery: existing barriers and current counter-strategies

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Keywords
diabetes; formulation; insulin; oral; tablet

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Abstract

Objectives The chronic and progressive nature of diabetes is usually associated with micro- and macrovascular complications where failure of pancreatic β-cell function and a general condition of hyperglycaemia is created. One possible factor is failure of the patient to comply with and adhere to the prescribed insulin due to the inconvenient administration route. This review summarizes the rationale for oral insulin administration, existing barriers and some counter-strategies trialled.

Key findings Oral insulin mimics the physiology of endogenous insulin secreted by pancreas. Following the intestinal absorption of oral insulin, it reaches the liver at high concentration via the portal vein. Oral insulin on the other hand has the potential to protect pancreatic β-cells from autoimmune destruction. Structural modification, targeting a particular tissue/receptor, and the use of innovative pharmaceutical formulations such as nanoparticles represent strategies introduced to improve oral insulin bioavailability. They showed promising results in overcoming the hurdles facing oral insulin delivery, although delivery is far from ideal.

Summary The use of advanced pharmaceutical technologies and further research in particulate carrier system delivery predominantly nanoparticle utilization would offer useful tools in delivering insulin via the oral route which in turn would potentially improve diabetic patient compliance to insulin and the overall management of diabetes.

Introduction

Insulin is administered to diabetic patients through the parenteral route. Most protein drugs are administered by injection due to their perceived low bioavailability if delivered by other routes. Insulin is injected subcutaneously, and this is usually associated with pain, trauma, distress, leading to poor patient compliance[1,2] which in turn cascades into a chain of undesired effects. The exogenous insulin is significantly able to reduce the rate of morbidity and mortality, although around 60% of diabetic patients fail to achieve long-term normoglycaemia,[3] presumably because of non-compliance issues resulting from needle phobia and complexity of the insulin treatment regimen. In effect, poor patient compliance would contribute to long-term poor glycaemic control and diabetic ketoacidosis and spark a series of macro- and microvascular complications.[4] Oral insulin was first trialled in 1922 with poor results.[5] Even to this day, a successful oral insulin preparation has been elusive, but is expected to dramatically improve patient outcomes. This review will address and discuss the following topics: insulin-related physiology and rationale for oral insulin use, basic challenges in oral delivery of insulin, some strategies trialled and finally mitogenic concerns of oral insulin.

Insulin-related physiology

After it is secreted from pancreatic β-cells into the portal vein, insulin is directly transferred to the liver where it is subjected to the hepatic first-pass effect where almost half of the polypeptide hormone experiences hepatic degradation.[6] Such an environment creates an insulin concentration gradient between hepatic portal and systemic

circulation, where the liver is exposed to insulin concentration twofold to fourfold higher than that observed in the peripheral systemic circulation.[6]

Available insulin preparations fail to mimic the endogenous insulin pathway where the injectable insulin analogues are delivered directly to the peripheral circulation, hence reversing the insulin concentration gradient in normal physiology, and as a result, insulin reaches the liver at much lower concentrations than those in non-diabetic individuals.[7] Diabetic patients treated with injectable insulin preparations are subjected to the side-effects of hyperinsulinaemia, weight gain and hypoglycaemic risks.[7]

A step towards normal physiology

The long-term use of the conventional insulin preparations, collectively called multiple daily insulin (MDI), results in poor glycemic control, risks of hypoglycaemia and needle phobia.[8,9]

On the other hand, treating the dawn phenomenon (early morning hyperglycaemia especially in pregnant patients) with conventional insulin preparations is quite complex and troublesome.[8,9] The aforementioned drawbacks raised the desire of diabetologists to get the basal insulin at a more physiological level through delivering predetermined continuous subcutaneous insulin infusion (CSII) or insulin pump which can also get programmed to deliver postprandial bolus insulin if required.[8,9]

In the late 1970s, insulin pumps were introduced and it was initially thought to be the most physiological way of delivering insulin subcutaneously to achieve near-normoglycaemia.

CSII offered more flexibility in daily life and that is probably why they were recommended during pregnancy or for preconception care and for type 1 diabetic children and adolescents.[8,9] New model insulin infusion pumps are light in weight (less than 0.5 kg), small in size, and contain replaceable insulin syringes or cartridges. The insulin is infused from the pump to the subcutaneous tissue through a simple and manually inserted catheter.[8]

Less invasive insulin delivery

Despite all the initial excitement, delivery of insulin through MDI or CSII still did not mimic the physiology of the endogenously secreted human insulin, where the insulin is reversely distributed between portal and systemic circulation which has driven researchers to develop less invasive and more physiological routes of insulin administration such as transdermal, inhaled, nasal, buccal, ocular, rectal and the highly preferred oral insulin.[7]

While stability requirements for formulation of a protein hormone as well as making sure of its therapeutic efficacy were taken into consideration by researchers, most of the developed formulations have failed to demonstrate satisfying bioavailability results.[7,10]

Oral insulin and rationale for its use

Physiological rationale

Orally administered insulin better replicates the normal physiological insulin pathway and leads to better glucose homeostasis.[11,12] After its absorption from the intestinal lumen, insulin is transported via the portal circulation to the liver (the ultimate target)[13] creating a high porto-systemic gradient. The oral route offers decreased levels of systemic insulin,[14] hence less hypoglycaemic episodes and weight gain problems (Figure 1).

The liver on the other hand is highly sensitive to insulin, and within few minutes, glucose production by the liver is blocked by a portal insulin concentration of 50 μu/ml in healthy objects and by around 100 μu/ml in diabetic patients.[15–17] Interestingly, oral insulin plays a significant role in protection of β-cells of the pancreas from autoimmune destruction.[18] Another study concluded the possibility of early insulin therapy to improve β-cell function and provided a chance for β-cell rest.[1,19] Insulin administered orally theoretically should be able to overcome the slow first phase release kinetics encountered with subcutaneous administration.[1,20]

Pharmaceutical rationale

It has been suggested that oral insulin offers induction of oral tolerance or immune modulating effect which is likely to help in prevention of diabetes.[1,21] The theory behind this phenomenon is that oral administration of a low dose of antigen alters responsiveness of systemic T cell to that antigen which seems to inhibit autoimmune diseases such as type 1 diabetes.[21] This effect has been experimented upon non-obese diabetic (NOD) mice treated with oral insulin, where peripheral T cells do not migrate to pancreatic islets[21] which might explain prevention of type 1 diabetes in NOD mice.[21] Despite its lower bioavailability, oral delivery of peptides showed high level of acceptance by patients with improved level of compliance[22] avoiding pain, discomfort, possible infections by injectable forms in addition to being less expensive. These are all factors that favour a switch to oral delivery of therapeutic peptides such as insulin.[22]

Challenges in oral delivery of insulin

The fundamental barrier to the oral delivery of proteins is the gastrointestinal tract (GIT). The GIT functions as

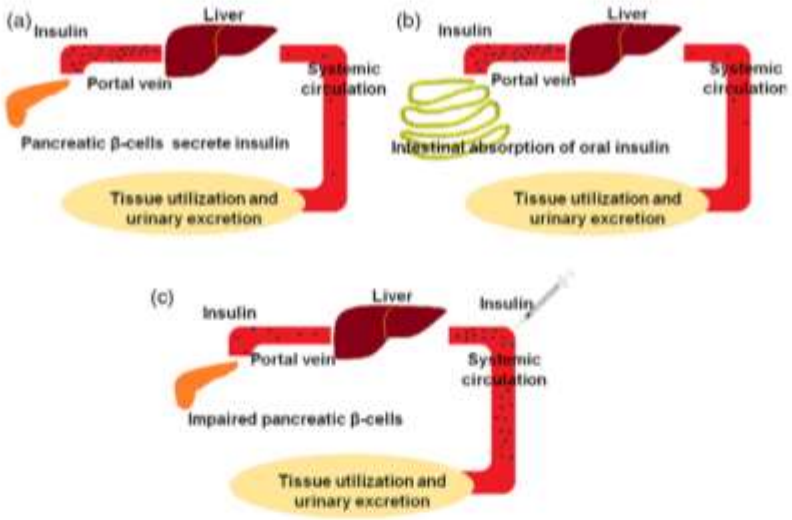

Figure 1 Endogenous versus administered insulin circulation pathways. (a) Endogenous insulin pathway under normal physiology; (b) orally administered insulin pathway; (c) injected insulin pathway in diabetic patients. [Colour figure can be viewed at wileyonlinelibrary.com]

an absorptive and protective organ. Active macromolecules have to undergo degradation into inactive components before they face the next absorption barrier, the tight epithelium. These natural mechanisms thus also act as a defence shield against environmental pathogens.[23–25]

The epithelial cell layer comprises different types of cells (Figure 2):

A Enterocytes: The most abundant, with dense well-ordered brush border, has absorptive function via transporting nutrients.[26,27]

B Goblet cells: The second most abundant in the epithelium which acts as a secretory reservoir of mucus.[26,27]

C Paneth cells, when exposed to bacteria, and antimicrobial proteins are secreted to kill the invading bacteria[26,27].

D Microfold cells (M-cells) of Peyer's patch characterized by less cytoplasmic lysosomes and lack of a mucous layer covering their surface. M-cells take up antigens and microorganisms from the lumen of the intestine and present these to the immune system resident in the mucosa.[28,29]

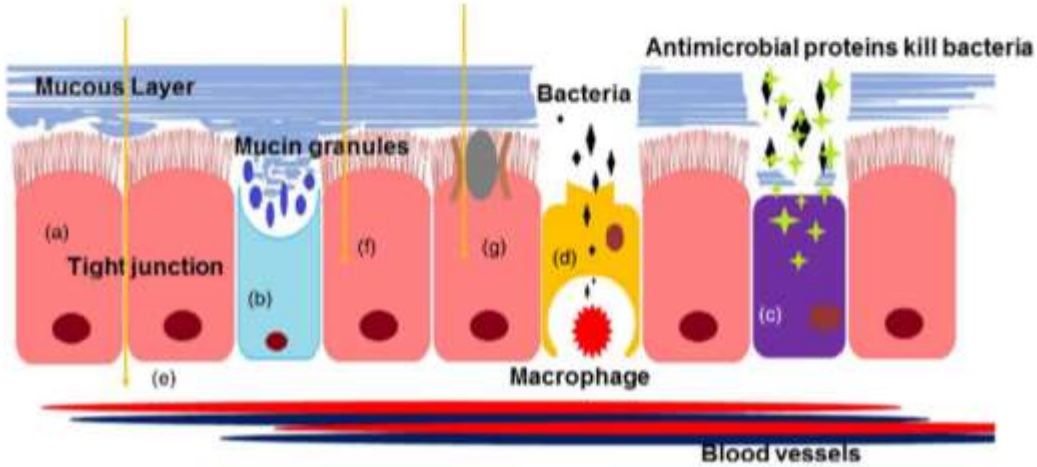

Figure 2 Different cell types of the healthy (normal) epithelial layer. a – enterocytes, b – goblet cells, c – Paneth cells, d – microfold cells (M-cells), e – paracellular transport, f – transcellular transport, g – carrier-mediated transport. [Colour figure can be viewed at wileyonlinelibrary.com]

E Paracellular route mainly for small hydrophilic molecules.
F Transcellular route is limited for passive diffusion of lipophilic substrates.
G Carrier-mediated transcellular absorption.

The tight junctions (TJs) between adjacent epithelial cells maintain the intactness of the intestinal epithelium structure. These TJs are composed of various transmembrane proteins such as claudins and occludins.[30,31]

Barriers to oral insulin

Existing barriers could be categorized into three main subtypes, namely physical, biochemical and formulation-based. (Table 1 summarizes possible barriers for oral insulin delivery and some of the strategies used).

Physical barriers

1 The mucous layer: Mucus is the first barrier which functions as both diffusional and enzymatic barrier encountered by polypeptides.[32,33] Mucus is continuously secreted and probably because of its negative charge, it filters out positively charged drugs and proteins by electrostatic interaction.[32–34]
2 Intestinal epithelium: The intestinal epithelium is composed of a single layer of columnar epithelial cells.[26–29] Passive diffusion is limited to lipophilic drugs less than 700 Da in molecular weight,[35] and because the molecular weight of insulin is 5800 Da,[36] this renders transcellular passive diffusion of insulin into cells very difficult.
3 Tight junctions: This dynamic structure is selectively permeable to small hydrophilic molecules (nutrients, ions

Table 1 Types of barriers facing oral insulin administration and counter-strategies

Barrier	Strategies introduced	Examples of agents used and/or contributing factor	Major findings	References
Mucus layer	Mucoadhesive systems	Chitosan, alginate, polyacrylic acid	Mucoadhesive polymers provide an intimate contact with the mucosa at the drug uptake site	[32,33,83,85]
		PLGA, thiomers	The strategy increases the residence time of the delivery system at the absorption site	
	Mucus penetration systems	Hydrophilic polymer coating such as (PEG), Virus-mimicking strategy (highly charged surface)	Efficient mucus permeation requires:highly densely charged surface, Hydrophilic shell and slightly negative surface charge	[87–89]
Intestinal epithelial cells and the transcellular route	Carrier-mediated transcellular absorption	Transferrin, immunoglobulins biotin, lectins, folate, vitamin B12	Due to their large molecular weight, conjugation of proteins to receptor-recognisable ligands will have the potential to improve cellular internalisation	[45,53,56,58]
	Cell penetrating peptides (CPPs)	Penetratin, oligoarginine, transportan, TAT	Perturbing cell membrane, endocytosis or even channel formation could be a possible mechanism of their action	[59–61]
	Absorption enhancers	EDTA, surfactants, fatty acids, ZOT	Perturb the cell membrane	[44,46,69,71]
Tight junction and paracellular route	Absorption enhancers		Selectively open tight junctions	
	Particulate carrier systems	Lipid suspension, liposomes, submicroemulsion, nanospheres, SLN, HDV-I, SNEDDS	They circumvent the harsh gastric conditions without affecting the bioactivity of macromolecules like insulin	[28,29,90,91,99–104]
			M cells of Peyer's patches play a unique role in the uptake of small particles	
Luminal pH	Colon-targeting and enteric coating	CODES, capsulin, ORMD-0801	Low level of luminal and brush border proteases	[56,73–79]
Enzymatic degradation	Protease inhibitors	Na-glycocholate, soya bean trypsin inhibitor, aprotinin, bacitracin, camostat mesilate, chicken and duck ovomucoids	Concerns about long-term use and protein malabsorption	[46,67,68]

PLGA, polylactic co-glycolic acid; PEG, polyethylene glycol; EDTA, ethylene diamine tetraacetic acid; ZOT zonula occludens toxin; SLN, solid lipid nanoparticle; HDV-I, hepatic direct vesicle insulin; SNEDDS, self-nanoemulsifying drug delivery system.

and certain drugs).[30,31] Solutes of molecular radius more than 15 Å are usually excluded from traversing this barrier.[31]

Biochemical barriers

1 Luminal pH: pH varies from highly acidic (1.2–3.0) in the stomach to slightly basic (6.5–8.0) in the intestine.[37] Such pH variation can cause pH-induced oxidation and deamination of protein drugs.[38]
2 Enzymatic degradation: Proteolysis starts at the stomach through the action of pepsin and continues throughout the intestine due to chymotrypsin, elastase and carboxypeptidases.[39] Presystemic degradation of proteins could also be attributed to cytosolic and membrane-bound enzymes of the enterocytes.[39]

Insulin is predominantly degraded by trypsin, α-chymotrypsin and carboxypeptidases in the mucous layer as well as intestinal lumen, with research also showing the existence of a specific insulin-degrading enzyme (IDE) on the brush-border membrane.[40,41]

Peptide drugs that do cross the intestinal epithelial barrier then undergo further degradation in the liver (hepatic first-pass metabolism).[11,42]

It has initially been thought that the inhibition of the IDE could be of value in preserving oral insulin formulations from enzymatic degradation, thereby improving its oral absorption and efficacy. A novel potent IDE inhibitor (6bK) has been revealed in a recent study which unfortunately did not improve oral insulin delivery.[43] IDE inhibition has been associated with increased amylin levels, which in turn slows the gastric emptying rate and improves glucose tolerance.[43] Thus, although the aim of improving oral insulin absorption was not attained, IDE inhibitors can still be of benefit in the treatment of T2DM patients.[43]

Formulation barrier

The fabrication method could be the last barrier in formulation of peptide drugs. Being a sensitive polypeptide hormone and any conformational changes to insulin structure would affect its biological activity.[44] Better ways for formulation of oral peptide drugs have been tackled by many scientists through various approaches, with two foci being the overcoming of intestinal hurdles and the ensuing low bioavailability.[45,46] Co-administration of enzyme inhibitors, addition of absorption enhancers or even slight modifications of the chemical structure of the protein are all among these techniques.[45,46] These emerging techniques (below) are basically aiming to improve pharmacokinetic and pharmacodynamic characteristics of the hormone.[45–47]

The suggested strategies trialled for oral insulin delivery can be classified into:

1 Chemical modification

The covalent attachment of polyethylene glycol (PEG) to therapeutic peptides has been utilized previously; the technology is called PEGylation.[48] PEGylation has been used to decrease the rate of clearance and improve the pharmacological and biological properties of peptides and eliminates the immunogenicity, allergenicity and antigenicity of insulin if compared with unmodified subcutaneous insulin.[49] (PEG-INTRON®) or PEG-modified α-interferon is an example of this technology which has been approved for hepatitis C treatment.[50]

The NOBEX Corporation worked on the same principal to develop hexyl insulin mono-conjugate-2 (HIM2). A short-chain PEG is linked to a lipophilic alkyl group to produce an oligomer. The amphiphilic oligomer was used to modify recombinant human insulin through attachment to lysine at position 29 of the B-chain[51] (Figure 3). HIM2 showed increased solubility, absorption, good stability against enzymatic degradation, although oral bioavailability was still ~5% and clinical studies showed efficacy in both type I and type II diabetic patients.[52,53] The research into PEGylated insulin technology has also been pursued by Biocon, a large Indian pharmaceutical company. Biocon took over NOBEX in 2005 and acquired the intellectual property of HIM2 where they developed the oral insulin candidate (IN-105) as a second-generation tablet.[54–56] IN-105 had an improved stability profile in GIT and enhanced absorption and in comparison with normal insulin, lower immunogenicity, lower mitogenicity and the same pharmacological action.[55]

Another example of structural modification has been pioneered by Emisphere's Eligen™. The technology comprises a reversible non-covalent interaction of insulin with low molecular weight carriers (200–400 Da).[45] These carrier molecules are organic/lipophilic in nature to improve the lipophilicity of insulin and facilitates passive transcellular diffusion without affecting cell membrane integrity or TJs.[45] Despite the relative fast absorption rate obtained from the pharmacokinetic data, Emisphere did not show a satisfactory bioavailability even in the presence of the large amount of carriers needed per dose.[45,55,57]

2 Targeting receptor/tissue

a Receptor-mediated endocytosis: It is thought that exploiting endogenous cellular transport systems could increase uptake of some drugs.[45,53] Cell membrane transporters have been utilized previously to transport relatively small therapeutics. Receptor-mediated endocytosis, on the other hand, offered better results in transport of macromolecules and proteins.[53] Lectins, transferrin, immunoglobulins, folate, cyanocobalamin (vitamin B12, vit B12), epidermal growth factor and

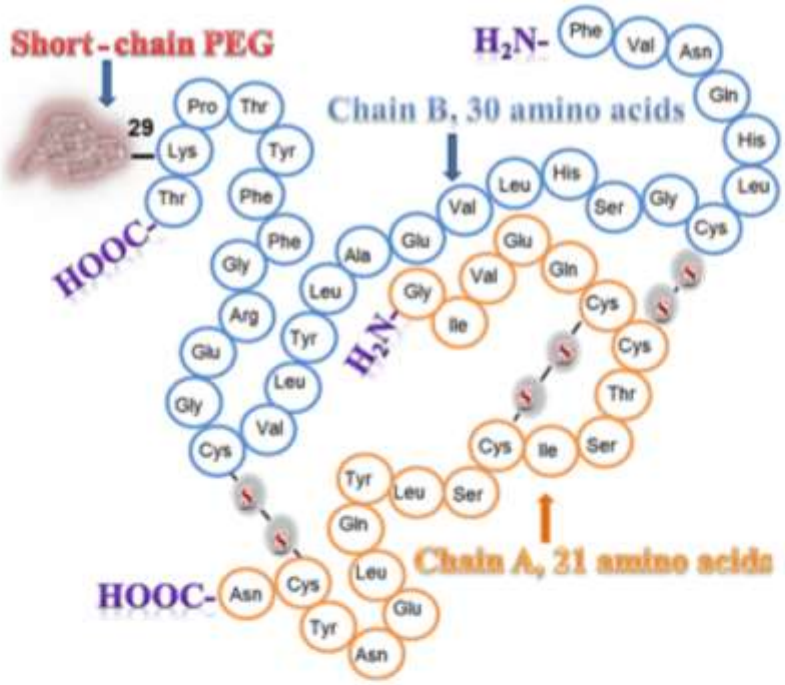

Figure 3 Hexyl insulin mono-conjugate-2 (HIM2). A short-chain polyethylene glycol (PEG) is attached to lysine at position 29 of the insulin B-chain. [Colour figure can be viewed at wileyonlinelibrary.com]

intrinsic protein complex are among receptor-recognizable ligands that have been covalently attached to a peptide to improve both specificity and intracellular delivery.[45,53] This principle has been adopted by Access Pharmaceuticals in development of CobOral™, a peptide-loaded dextran nanoparticle coated with cobalamin. Likewise, Apollo Life has developed a carbohydrate-based nanoparticle coated with Vit B12 called Oradell™ [56]. Vit B12-coated dextran nanoparticles loaded with insulin showed significant prolonged hypoglycaemic effect in a STZ diabetic rat model.[58]

b Cell-penetrating peptides (CPPs): CPPs is a relatively recent strategy in its early stage which has the potential for effective delivery of liposomes, nanoparticles, small molecules, as well as peptide drugs.[59] The technique is based upon using a class of short peptides such as penetratin, oligoarginine, transportan and HIV-1 transactivator of transcription (TAT).[60] No clear mechanism of translocation and internalization of their cargoes into the cytoplasm has emerged, although direct perturbation of the cell membrane, endocytosis or channel formation is possible mechanisms that have been hypothesized.[61] CPPs showed no toxicity or side-effects with in-vivo use and the transport of insulin/TAT conjugate across Caco-2 cells showed significant improvement.[53,62]

3 Formulation/pharmaceutical technologiesThe use of these formulation technologies/strategies does not include physicochemical modification of the peptide to withstand gastric conditions. In fact, shielding insulin by encapsulation in an enteric-coated dosage form, co-administration with protease inhibitor or addition of permeation enhancers have been deployed with varying degrees of success. Detergents, fatty acids or bile acid salts are among the absorption enhancers used to facilitate the internalization of the hormone and overcoming the intestinal conditions to some extent.[44–46,56] Examples of formulation-related technologies are as follows:

a Gut-associated lymphoid tissues: Gut-associated lymphoid tissues are best represented by Microfold cells (M-cells) of Peyer's patch. M-cells are responsible for antigen sampling and microparticulate uptake smaller than 10 μm.[63,64] An uptake that has been used before for mucosal vaccine delivery through the per-oral route.[65] Nanoparticles of chitosan showed a significant protection of encapsulated insulin against enzymatic degradation, and such nanoparticles were able to cross the epithelium through Peyer's patches.[65,66]

b Protease inhibitors: As insulin is degraded by trypsin, α-chymotrypsin and elastase, inhibition of the activity

of these enzymes would decrease presystemic insulin degradation and improve its absorption.[46] Long-term use of these inhibitors is still an issue which could result in protein malabsorption due to disturbed protein digestion. Protease inhibitors could also be associated with systemic toxicity.[44] To this end, the enzyme inhibitory effect of Na-glycocholate, aprotinin, bacitracin, soya bean trypsin inhibitor and camostat mesilate has been compared. Co-administration of these agents with insulin directly into isolated intestines of normal rats resulted in improvement of the bioavailability of insulin. The effect was more predominant in the large intestine than the small intestine where Na-glycocholate, bacitracin and camostat mesilate showed better results.[67] Chicken ovomucoid (Ckovm) and duck ovomucoid (Dkovm) also showed protective effect against trypsin and α-chymotrypsin degradation of insulin. This study concluded that Ckovm inhibited trypsin-mediated degradation at 1 : 1 ratio, while Dkovm showed 100% protection against trypsin and α-chymotrypsin for 60 min at 1 : 2 ratio of enzyme inhibitor.[68]

c Absorption enhancers/permeation enhancers (PEs): PEs are a group of agents that promote absorption of therapeutics through perturbing the cell membrane to improve transcellular transport or by selective action on TJs to enhance paracellular permeability. Decreasing mucous fluidity, change in membrane fluidity and leakage of proteins by opening TJs are possible mechanisms suggested for their action.[46,69] However, the long-term use of PE is a controversial issue, as some researchers are concerned about toxic effects upon prolonged use.[46,69] However, others argue that their use is by far the most advanced technology in oral peptide delivery. They reckon clinical studies showed good safety profile and achieved consistency of bioavailability for peptides less than 10k Da.[70] Bile salts, ethylene diamine tetraacetic acid, surfactants, fatty acids and zonula occludens toxin (ZOT) are examples of permeation enhancers commonly used to improve oral peptide bioavailability.[44,46,69] In-vivo results showed that ZOT increased insulin oral absorption 10-fold from rabbit ileum and jejunum and no effect was noticed in colon.[71]

d Site-specific deliveryColon targeting has been interestingly used to deliver therapeutic peptides and non-peptides. Low level of luminal and brush-border proteases compared to duodenum and jejunum encouraged scientists to the use of colon targeting to circumvent harsh gastric conditions.[56] Insulin has been encapsulated by pH-sensitive materials to control its release and that showed improved oral bioavailability. Polyacrylic-coated gelatin capsules loaded with insulin have been studied in rats and showed significant drop in the blood glucose level compared to intraperitoneal injection.[72] An interesting colon-specific drug delivery system, CODES™, is based on a core tablet coated with different polymeric layers. CODES showed sustained release of insulin in the colon of dogs. The formula incorporated the hormone with meglumine (a pH adjuster), citric acid (insulin solubilizer), Na-glycocholate (a permeation enhancer) along with polyethylene oxide.[73,74] Capsulin™, a product of diabetology, is an enteric-coated capsule loaded with dry powder mixture of insulin, permeation enhancer and solubilizer. The ingredients of the capsule are generally safe pharmacopoeial excipients.[75–77] Sixteen T2DM patients were subjected to 11 days apart, two isoglycaemic glucose clamp studies in which they compared the pharmacokinetic and pharmacodynamic outcomes of both (150U or 300U) of orally ingested Capsulin and 12U of subcutaneously injected regular human insulin (Actrapid),[77] and Capsulin showed good gastric stability where the dissolution starts in jejunum, reasonable safety profile with well-tolerated statistically significant hypoglycaemia for over 6 h.[75–77] Another pharmaceutical company, Oramed, has also developed an enteric-coated capsule (ORMD-0801) containing 8 mg insulin which has been marketed for type I and type II diabetic patients.[78,79] The pharmacokinetic and pharmacodynamic characteristics of ORMD-0801 capsules were evaluated through phase IIa when 2 capsules of the product were given to type 1 diabetic patients.[76] The study concluded the safety and biological activity of the product when given before meals, and it is eliminated in around 5 h.[76] Phase IIb study has been conducted in type 2 patients to also determine how safe, tolerable and effective ORMD-0801 is during 6-week period when compared to placebo.[76] ORMD-0801 showed remarkable reduction in blood glucose level, increased plasma insulin level as well as reduction in C-peptide.[78,79] A promising approach in oral peptide delivery is the introduction of superporous hydrogel (SPH) and superporous hydrogel composite (SPHC) in targeting proteins to a specific site of the intestine.[80] The new core and shuttle delivery system were developed to mechanically localize the peptide drugs in the intestinal lumen.[80] The drug is incorporated in the core which is either attached to or embedded in the enteric-coated shuttle or conveyor of SPH and SPHC.[80] The novel delivery system tried to achieve an in-vitro double-phase time-controlled release model and showed partial inactivation of trypsin due

to calcium binding and enzyme entrapment within the polymeric system.[80] Interpenetrating polymeric networks of superporous hydrogels (SPH-IPNs) using poly (acrylic acid-co-acrylamide) and O-carboxymethyl chitosan have been trialled to estimate insulin transport across rat intestine and colon.[81] No conformational changes to insulin or alterations to its oral bioactivity in terms of hypoglycaemic action have been recorded using (SPH-IPNs) following administration to healthy animals.[81] When it is compared to the subcutaneous route, insulin-loaded SPH-IPNs delivery system achieved around 4% pharmacological availability and the system partly inactivated trypsin and α-chymotrypsin.[81] This system improved paracellular insulin transport through excised rat intestine and across CaCo-2 cell monolayers by 4.2- and 4.9-fold, respectively, via transient opening of epithelial TJs, and it can be retained in rat intestine for longer compared to the powdered SPH-IPN.[82]

e Bioadhesive (mucoadhesive) systems and mucus penetrationThe mucoadhesive properties of some polymers have been exploited to prolong the residence time of the drug at its absorption site by increasing the contact with mucosa which in fact increases the concentration gradient of the drug.[83] Some of these polymers in addition to their mucoadhesive characteristics have a dual role as protease inhibitors or permeation enhancers.[83,84] A new generation of mucoadhesives was formulated via the introduction of thiomers or thiolated polymers. Thiomers have dramatically improved the mucoadhesive properties of anionic polymers such as polyacrylic acid and alginate or cationic polymers such as chitosan.[85] This improvement is attributed to the formation of a stronger disulphide bond which can improve the mucoadhesive properties 140-fold compared to unmodified polymers.[85] Chitosan-4-thiobutylamidine insulin-loaded tablets showed controlled release in non-diabetic rats for over 8 h.[86] Mucus penetration is another technology introduced to overcome the dynamic upstream mucus barrier resulted from its rapid turnover kinetics.[87–89] Efficient mucus penetration of an insulin carrier requires highly densely charged surface (virus-mimicking strategy), coating of insulin carrier with hydrophilic mucus inert polymer and neutral to slightly negative charged surface (to minimize electrostatic interaction with mucus).[87] PEGylation is one of the approaches that used PEG coat as hydrophilic mucus inert polymer to facilitate mucus penetration of insulin-loaded carriers.[88] Virus-mimicking strategy has been combined with PEGylation technique in a study for oral insulin delivery as a model of utilizing two different concepts for mucus penetration systems.[87] The specific design, PEG molecular weight requirements, limited cellular uptake and induction of anti-PEG antibodies might be considered potential limitations of PEGylation technology.[88] *N*-(2-hydroxypropyl) methacrylamide (HPMA) is another hydrophilic mucus inert polymer, and HPMA has been recently used to coat insulin-loaded N-trimethyl chitosan (Ins-TMC) nanocarriers. The novel approach utilized the mucus penetration characteristics of HPMA to deliver the mucoadhesive (Ins-TMC) nanocarriers. Upon oral administration to diabetic rats, remarkable hypoglycaemia has been noticed with a potential success of oral insulin delivery.[89]

f Particulate carrier systemFormulation of drugs with colloidal particulate carriers has been widely used to improve peptide delivery. Submicroemulsion, lipid suspension, liposomes, polymeric micro- and nanoparticles and polymeric micelles are good examples of this.[90,91] These approaches seem to circumvent the existing barriers and harsh gastric conditions for oral peptide delivery demonstrating that good release profiles and the bioactivity of hormones such as insulin could be well maintained too.[90,91] Encapsulation of insulin in the form of nanospheres facilitates its absorption across the intestinal epithelium. The nanospheres are taken up in tissues such as liver where polymer degradation occurs.[52] Insulin nanospheres could then function as minor reservoirs for insulin inside the targeted organ (the liver).[52,64,92] Chitosan microspheres loaded with insulin have been studied too. Relative increase in insulin pharmacological bioavailability has been observed when optimal particle size of the microspheres is selected.[93]

A novel insulin-loaded chitosan phthalate microsphere formulation was found to sustain the plasma glucose of rats for at least 16 h at the prediabetic level with improved oral bioavailability.[94] Another novel solid-in-oil-in-water (S/O/W) emulsion has been formulated with reasonable efficacy in oral delivery of peptides and proteins. This technique is used to encapsulate insulin and showed a pH-responsive release pattern simulating GIT conditions.[95] In an interesting technique based on a low shear reverse micellar approach, a surfactant mixture was prepared by mixing didoceyldimethylammonium bromide (a surfactant) and propylene glycol (a co-surfactant). Triacetin (the oily phase) was then added to the surfactant mixture at the optimized ratio of 1 : 3, respectively. The aqueous insulin solution was eventually added to the previous mixture while stirring to make a final concentration of 20% v/v aqueous phase in the mixture.[96] The transparent microemulsion produced was able to entrap insulin to

considerable extent (~85%) without affecting its conformational stability. In-vivo results showed 10-fold improvement in bioavailability compared to plain insulin solution upon oral administration to healthy rats.[96]

Limited success since 1976 has been achieved using liposomes for oral delivery of peptides.[97,98] The unsatisfactory bioavailability with liposomes could be attributed to their instability or limited absorption in GIT conditions, although the natural chemicals (phospholipids) used for formulation are a great advantage.[97,98] However, a liposomal insulin formulation known as hepatic direct vesicle insulin (HDV-I) has been successfully delivered orally.[55,99,100] The novel vesicles are composed of (<150 nm diameter) liposomes which contain insulin attached to a specific proprietary molecule known as hepatocyte-targeting molecule in an attempt to replicate normal insulin physiology.[55,99,100] HDV-I is formulated as an oral gel capsule, stable at low pH of blood and resists gastric degradation with high biopotency.

The solid lipid nanoparticle (SLN) is another example of lipid-based nanoparticulate carrier that has been developed as an alternative to polymeric nanoparticle systems.[101,102] SLNs show good biocompatibility, biodegradation, sustained release of the incorporated molecules and amenability to large-scale production.[101,102] Insulin-loaded PEG-stearate-coated lipid nanoparticles offer good protection of the encapsulated insulin when in contact with gastrointestinal fluids compared to non-coated lipid nanoparticles.[103]

Self-nanoemulsifying drug delivery systems (SNEDDS) are recent techniques which combine the advantages of nanotechnology and lipid-based drug delivery.[104] The strategy was introduced to improve the formulation of not only the lipophilic drugs but poorly water-soluble therapeutics as well.[104] SNEDDS were initially introduced to overcome the drug solubilization and bioavailability issues of biomolecules due to their ability to enhance droplet surface area, protect from gastric enzymes, improve permeation, alter gastric retention time and sometimes drug mucoadhesive properties.[104] Current applications of SNEDDS include improving drug stability, controlling the drug release, increasing drug loading capacity (<25 mg to >2 g) and specific tissue targeting which can be of potential in lymphoma, leukaemia and autoimmune diseases.[104,105]

In a formulation development study, SNEDDS was produced using a solid dispersion technique in a trial to formulate a non-invasive delivery carrier for therapeutic proteins and it showed promising results.[106] In an attempt to improve oral insulin bioavailability, researchers combined the technology of a multifunctional polymeric system using thiolated chitosan and SNEDDS technology to produce (80–160 nm) spheres with remarkable insulin entrapment efficiency.[107] In-vitro release profile from insulin/thiolated chitosan SNEDDS significantly increased and serum insulin level showed pronounced increase in in-vivo studies when compared to oral insulin solution.[107]

SNEDDS loaded with insulin-lecithin complex facilitated the transport of insulin phospholipid complex across Madin–Darby canine kidney (MDCK) cell monolayer, with no cytotoxicity.[108] Oral administration to diabetic Wistar rats resulted in remarkable hypoglycaemia with relative bioavailability up to 7.15%.[108] In a recent study, a novel SNEDDS was developed to facilitate the mucus permeation of oral insulin,[109] based upon hydrophobic ion pair of insulin with dimyristoyl phosphatidylglycerol (DMPG) to form insulin/DMPG complexes.[104,109]

The technique relied on the fact that insulin acquires net positive charge at low pH which facilitates its conjugation with anionic amphiphilic partners (phospholipids, surfactants and fatty acids) through ionic complexation.[104,109] In addition to the good permeation characteristic, insulin/DMPG complex-loaded SNEDDS provided protection from GIT enzymes and prevented initial burst release of insulin which demonstrates a potential for oral insulin delivery.[104,109]

Nanoparticle architecture and polymer choice in oral insulin delivery

Nanotechnology using polymers has been one of the most interesting approaches in formulation of peptide therapeutics, and to this end, insulin formulated using nanoparticle technology showed increased cellular uptake and transport across Caco-2 cells.[110] It has been the focus of many researchers to optimize nanoparticle carriers to protect macromolecules from gastric enzymes as well as improve intestinal permeation.[56] Different studies have been carried out using polymer-based nanoparticles for oral insulin delivery using different polymers and their derivatives, and the results have been quite promising[111] (Table 2).

Generally, an ideal polymeric carrier should be biodegradable, biocompatible and able to prolong intestinal residence time after resisting gastric pH gradient and enzymes.[112]

Based on nature, polymers utilized in fabrication of insulin nanoparticles are either of natural or synthetic origin.[111,112] Gelatin and casein, examples of natural protein polymers, have been used to prepare insulin-loaded nanoparticles for per-oral delivery with promising results and good safety profile.[112] Natural polysaccharide polymers have also been used extensively in nanoparticle design of insulin carriers due to their non-toxic characteristics. Examples of these polysaccharides are chitosan, alginate, dextran, starch and pectin.[112]

The use of synthetic polymers in formulation of oral insulin has been advantageous in sustaining the release of insulin over a period of days to several weeks compared to natural polymers.[111] Examples of these synthetic polymers

Table 2 Examples of polymeric nanocarriers used for oral insulin formulation and their efficacy

	Nanoparticle size (nm)	Animal model	Dose (IU/kg)	Comment	References
Poly lactic-co-glycolic acid (PLGA)	200	STZ diabetic rats	20	Up to 90% insulin entrapment efficiency 7.7% relative bioavailability compared to subcutaneous route Prolonged effect (12 h) 57.4% reduction in plasma glucose level within first 8 h	[129]
Poly lactic acid (PLA-F127-PLA)	56	Diabetic mice	50	Insulin loading capacity of 0.08% weight Hypoglycaemia over 23 h Highest blood glucose reduction (from 18.5 to 4.5 mmol/l) after 5 h	[130]
Poly (Ɛ-Caprolactone)/ Eudragit RS	700	Diabetic rats	50	97.5% insulin encapsulated with 70% *in vitro* release over 24 h Peak hypoglycaemia at 8 h (−52% for nanoparticles loaded with Aspart insulin)	[131]
Dextran + Vit B12	150–300	STZ diabetic rats	20	Prolonged antidiabetic effect (54 h) Blood glucose reduction of 70–75% 29.4% relative bioavailability compared to subcutaneous injection	[58]
Cetyl palmitate-based solid lipid nanoparticle (SLN)	350	Diabetic rats	50	>43% association efficiency Considerable hypoglycaemia over 24 h Relative bioavailability of 5.1% compared to subcutaneous route	[132]
Chitosan	250–400	Diabetic Wistar rats	21	Prolonged hypoglycaemia for >15 h Relative bioavailability for up to 14.9% compared to subcutaneous route	[65]
Chitosan/Alginate	750	STZ diabetic rats	50 and 100	Association efficiency > 70% >40% reduction in serum glucose level for >18 h Relative bioavailability of: 6.8% for 50 IU/kg and 3.4% for 100 IU/kg compared to subcutaneous injection	[133]
Chitosan/poly γ-glutamic acid	218	Diabetic rats	30	71.8% loading efficiency Significant hypoglycaemia for not less than 10 h Relative bioavailability for up to 15.1% compared to subcutaneous route	[134]

are polylactic acid, polylactic co-glycolic acid and poly (Ɛ-caprolactone), and they all have hydrophobic nature.[111,113] The internalization and uptake process of nanoparticles via the transcellular pathway is dependent on different factors such as surface charge, mucoadhesive properties and particle size.[114,115] Due to their biocompatibility and low toxicity, gold nanoparticles have been used to deliver insulin orally and intranasally. Hypoglycaemia has been recorded when given to diabetic Wistar rats.[7,116]

Cytotoxicity test as well as immunological response could be a requirement to assess the safety of nanoparticles. Biodegraded nanoparticles if accumulating inside the cells can cause intercellular changes in terms of organelle integrity which could lead to severe toxicity.[117] It has been suggested to consider the nanomaterial waste as unsafe due to their behaviour at the cellular and subcellular levels.[118] Inadequate data about the potential toxicity of nanomaterials to cellular and subcellular organelles are the main reason for the limited use of many nanoparticles.[119]

The surface of nanoparticles exhibits high reactivity towards proteins, biomolecules and biological fluids in the cell interior which cause these subcellular structures to associate with the nanoparticle surface in a phenomenon called bimolecular corona formation.[120] The design, chemical composition, size, shape, solubility, surface functionality and aggregation state of nanoparticles are all important factors in controlling and determining not only the cellular and subcellular transport of nanoparticles but their biokinetics and their biodistribution as well.[118,120,121]

Postinternalization of nanoparticles inside the cells, their surface charge can potentially determine the targeted subcellular organelle (lysosomes, cytoplasm, mitochondria or even the nucleus).[120,122] Possible cellular toxicity of NPs is best explained through the reactive oxygen species and oxidative stress model[120,123] which creates a vicious environment such as perturbation of mitochondrial activity, induction of pro-inflammatory effects and nuclear uptake.[118,121,123] All these effects could eventually lead to

cell injury and death[118,121,123] (Figure 4). This perturbed activity caused by NPs has been labelled by some scientists as latent toxicity due to modulation of otherwise normal cellular activity.[119]

Recent developments in oral insulin delivery

A novel intestinal insulin device has been developed from a mixture of mucoadhesive polymers in the form of 13-mm disc. The disc has been coated three times with ethyl cellulose leaving one surface uncoated to ensure unidirectional release of its insulin load. The mucoadhesive disc along with dimethyl palmitoyl ammonio propanesulfonate (a permeation enhancer) has been placed in a capsule (enteric coated with Eudragit L100) for intestinal delivery. After it has been liberated from the capsule shell, the insulin-loaded device completely released its protein content within 4 h with promising results in the animal model.[124]

The same technique has been exploited in the development of 500 μm-sized intestinal insulin patch. The novel micropatch (either coated or uncoated) has been encapsulated the same way with dimethyl palmitoyl ammonio propanesulfonate in addition to citric acid (a non-specific protease inhibitor). The small size of the patches delivered in the enteric-coated capsule offered mucoadhesion to larger surface area of the intestinal mucosa and resulted in a significant hypoglycaemia to male Wistar rats following oral administration.[125]

In a proof-of-concept study, insulin-loaded dodecylamine-graft-γ-polyglutamic acid micelles were developed and cross-linked with trimethyl chitosan (TMC) in the form of nanoparticle complex. The novel self-assembled polyelectrolyte complex nanoparticles are further modified to improve their affinity to the epithelium. This has been achieved by attachment of goblet cell targeting peptide (CSKSSDYQC) to TMC coat. Oral administration of the developed targeted nanoparticles has a relative bioavailability of 7.05% with prolonged hypoglycaemia in diabetic rats.[126]

In a novel attempt to modify the hydrophobic nature of SLNs and improve insulin encapsulation efficiency, methocel, a hydrophilic polymer has been incorporated into the internal phase of w/o/w double emulsion based SLN.

Methocel-lipid hybrid nanocarrier (MLN) has been prepared by emulsification solvent evaporation technique. In addition to the improved encapsulation efficiency, MLN also offered extra stability to entrapped insulin with the potential for a promising nanocarrier for oral peptide delivery.[127]

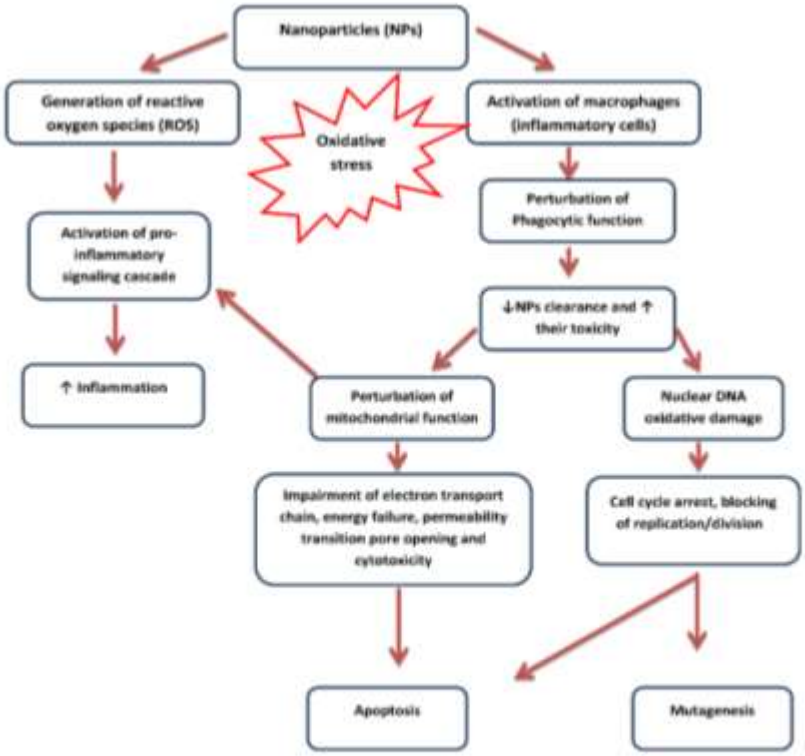

Figure 4 Currently known cytotoxic effects of nanoparticles. [Colour figure can be viewed at wileyonlinelibrary.com]

In a recent study, polyelectrolyte complexation of two natural polymers chitosan and carboxymethylated iota-carrageenan has been employed in the production of insulin-loaded nanoparticles. The carboxymethylation of iota-carrageenan as well as nanoparticle formulation has been optimized through the computational model, response surface methodology based on Box–Behnken design. Insulin has pH-responsive release from chitosan/carboxymethylated iota-carrageenan nanoparticles with good protection against gastric conditions.[128]

Due to the growing interest in developing starch-based nanocarriers, short-chain glucan, SCG (a debranched starch), has been employed in manufacture of insulin–SCG nanoparticles. The addition of cross-linker, proanthocyanidins (PAC) (isolated from red peanut skins) improved the insulin encapsulation efficiency. Insulin SCG/PAC nanoparticles exhibited gastric stability and significant hypoglycaemia for 8 h following oral administration in diabetic rat model.[129]

In a proof-of-concept study, insulin-loaded selenium nanoparticles were formulated by ionic cross-linking reduction technique. Insulin-selenium nanoparticles were produced *in situ*. Addition of sodium selenite and glutathione onto insulin/chitosan complex caused reduction in selenium ion and precipitation of selenium onto insulin/chitosan complex. The produced selenium nanoparticles have good insulin encapsulation efficiency as well as gastric stability. Oral administration of the developed nanoparticles showed remarkable hypoglycaemia in diabetic and non-diabetic rats. The study suggests that selenium can potentiate the antidiabetic effect of insulin and serve as a new oral nanocarrier for the hormone. The study also concluded that insulin-loaded selenium nanoparticles could have the potential to alleviate diabetes-associated oxidative stress and improve pancreatic β-cell functions.[130]

In a recent study based on core-shell nanoparticle technology, insulin has been encapsulated in polyurethane-alginate core with an outermost shell of chitosan. Insulin-loaded polyurethane-alginate/chitosan nanoparticles controlled the release of the entrapped hormone and the mucoadhesive nature played an important role in the hypoglycaemic effect noticed on fasted Swiss albino mice. The histopathological studies suggest the safe use of the developed nanoparticles to deliver insulin orally.[131]

In a recent study, hydrophobic ion pairing technology has been employed to complex insulin with sodium deoxycholate. Insulin-sodium deoxycholate complex improved the liposolubility of insulin and enhanced encapsulation efficiency of insulin complex in poly(lactide-co-glycolide) (PLGA). Insulin-loaded PLGA nanoparticles were prepared by emulsion solvent diffusion method. PLGA nanoparticles are then spray-dried with hydroxypropyl methyl cellulose phthalate (a pH-responsive polymer) for intestinal delivery. The new multifunctional composite microcapsule represents a promising carrier for oral peptide delivery.[132]

Insulin, insulin-like growth factor (IGF-1) and their receptors

Insulin, to mediate its pharmacological effects, has to bind to insulin receptor (IR).[133] IR belongs to a class of receptors known as tyrosine kinase superfamily which comprises IR as well as insulin-like growth factor-1 receptor (IGF-1R).[134] IR and IGF-1R show significant structure homology, and their configurations are almost identical.[134–136]

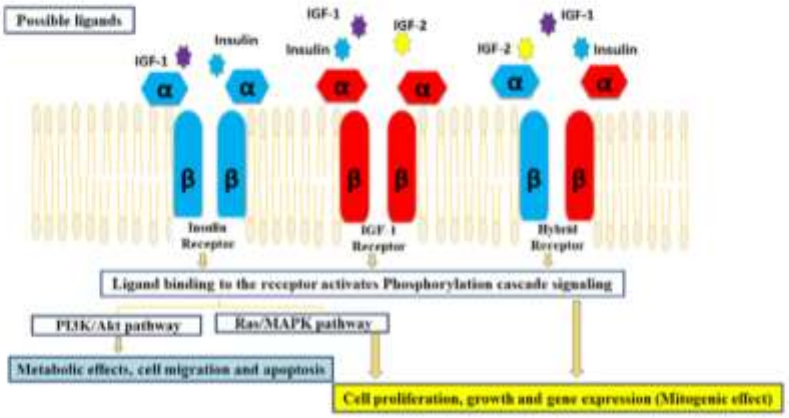

Figure 5 Simplified diagram of insulin and insulin-like growth factor-1 (IGF-1) signalling pathways. In summary, both are critical to cell health and survival, and there is significant overlap between the two. PI3K, phosphatidyl inositol 3 kinase, Akt; PkB, protein kinase B; Ras, rat sarcoma protein; MAPK, mitogen-activated protein kinase. [Colour figure can be viewed at wileyonlinelibrary.com]

IR and IGF-1R are located on the cell surface, and both exhibit heterotetrameric architecture.[134–136] Each receptor is composed of two extracellular ligand binding α-subunits and two transmembrane β-subunits linked by disulphide bonds.[134–136] Another hybrid receptor has been identified (IR/IGF-1R) which is composed of one α-subunit and one β-subunit from IR which heterodimerize with similar complementary units from IGF-1R.[134]

IR/IGF-1R, the hybrid receptor, exhibits higher affinity towards IGF-1 than insulin and tends to mediate IGF-1 mitogenic responses.[134] Insulin-like growth factors (IGF-1 and IGF-2) are polypeptide ligands that share fundamental structure homology with pro-insulin, and their primary function is to stimulate cell growth, cell differentiation, cell proliferation and cell survival.[133] GIT, liver and other tissues of the body synthesize and secrete IGF-1 and IGF-2, and interestingly, the GIT itself is considered as potential target organ of their mitogenic actions.[136] The close propinquity between insulin and IGF-1 as possible ligands and IR and IGF-1 receptor enables insulin and IGF-1 to bind to each other's receptor and not only to their cognate receptors.[135] Insulin can potentially bind to IGF-1R as well as IGF-2R with much lower affinity. On the other hand, IGF-1 is able to bind to both IGF-1R and IGF2R and weekly to IR. However, IGF-2 can only bind to its receptor and IGF-1R with no affinity towards the IR.[133] IR predominantly serves in expressing the metabolic intracellular pathways while IGF-1R primarily initiates cell growth.[134]

It has been reported that some mitogenic effects have been mediated through the IR, whereas IGF-1 has contributed somewhat to this.[136] The growth-promoting effect of insulin on cell proliferation and differentiation has been shown to be dose-dependent.[134,137] Human IGF-1, on the other hand, increased the sensitization to insulin and has improved fasting and postprandial glycaemia in some clinical studies on type 2 diabetic patients.[134] So, the ligand binding (either insulin or IGF-1) to IR or IGF-1R would potentially activate trans-autophosphorylation and the same downstream signalling pathways for cell proliferation, cell differentiation, anti-apoptotic effect as well as metabolism.[134,135]

Activation of either IR or IGF-1 receptor triggers a series of signalling transduction cascade pathways which can generally be categorized into:

A Rat sarcoma protein (Ras)/mitogen-activated protein kinase (MAPK) pathway which significantly mediates mitogenic action and gene expression[133,138] or

B Phosphatidyl inositol 3 kinase (PI3K)/protein kinase B (Akt), a pathway that broadly mediates insulin metabolic effects (glucose homeostasis) in addition to cell migration and anti-apoptotic action[133,138,139] (Figure 5).

Insulin binding to IR has been reported to stimulate colon cancer cells through the PI3K/Akt pathway.[139] Regardless of insulin type or origin, a potential mechanism of insulin mitogenicity has been explained in a condition of hyperinsulinaemia associated with insulin resistance.[140] This unique effect of insulin is mediated through IR and not thorough IGF-1 receptor.[140] This mechanism is mediated via activation of farnesyltransferase and increases the membrane-anchored farnesylated Ras for further activation by insulin as well as other growth factors.[140] Another possible, yet indirect mitogenic effect of insulin could be achieved by the stimulatory effect of insulin on pituitary gland to produce growth hormone which directly governs IGF-1 production by liver.[133,136] Long-term use of oral insulin for a chronic disease is still questionable, and being a growth promoter, oral administration of insulin has the potential for inducing mitogenic changes in the gut mucosa which has raised concerns in some scientists.[66,113]

Conclusion

Non-invasive hormone and peptide administration has been an interesting challenge for a long time in the pharmaceutical drug delivery field. The limited administration routes and low oral bioavailability of insulin have attracted many scientists since 1922 to tackle and improve more physiological and non-invasive insulin delivery options such as buccal, nasal and ocular delivery routes. Oral delivery of pharmaceuticals especially hormones is of higher preference among all other administration routes. The convenience of administration by patients themselves is a major factor for consideration. In line with this, physicians and clinical diabetologists favour the oral route in diabetes treatment to ensure patient compliance and to achieve the therapeutic targets.

There is a vast body of information in the literature outlining different techniques and strategies to improve oral insulin bioavailability, including proof-of-concept studies indicating the potential feasibility of successful oral insulin administration. The initial promising results obtained from these studies have been an impetus to undergo phase I and II clinical trials of some oral insulin products. The introduction of some innovative approaches such as mucoadhesive polymers, absorption enhancers, protease inhibitors as well as particulate carrier systems has boosted the scope of research in delivering insulin orally by counteracting the naturally existing hurdles and harsh conditions of the GIT. Funding, on the other hand, has always been a paramount limitation to obtain thorough pharmacokinetic and pharmacodynamic data in animals and humans and possible long-term side-effects of the newly introduced oral insulin candidates.

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Novel analytical method for simultaneous determination of the binary mixture of the antidiabetic drugs, metformin and gliclazide.

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Introduction

- Metformin is the mainstay antidiabetic agent since 1950s and has been identified as the first line therapy according to the international therapeutic guidelines for type II diabetes.
- Gliclazide is a second-generation sulfonylures that has been recommended by some guidelines as the second line therapy for type II diabetes.
- Metformin is a water-soluble basic biguanide while gliclazide is a water-insoluble acidic in nature.
- Simultaneous analytical HPLC method of the binary mixture is quite challenging due to opposite chemical natures of both analytes.

Aim

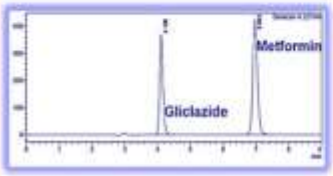
- To develop and validate an efficient isocratic HPLC method for the simultaneous determination of gliclazide and metformin on the same chromatographic run.

Method

- A simple mobile phase that consisted of 20 mM ammonium formate buffer (pH 3.5) and acetonitrile (45:55, v/v).
- The chromatographic column used for the optimized separation method is Altima CN (250 mm x 4.6 mm x 5µ).
- Detecton of gliclazide and metformin peaks was achieved by UV detector at wavelength 227 nm.
- Validation of the new method was done in accordance with the International Conference of Harmonisation (ICH) guidelines Q2(R1), Geneva, Switzerland 2005.

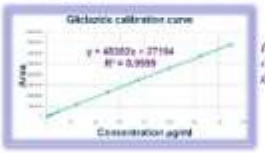
Results

- Two sharp, well separated gliclazide and metformin peaks attained in less than 10 minutes chromatographic run.



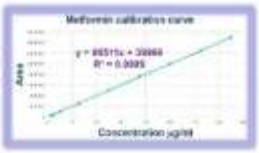
Typical chromatogram of the binary mixture of gliclazide and metformin 30µg/ml each.

- The new chromatographic method developed is specific for the analytical determination of gliclazide and metformin.



Linearity curve of gliclazide

- The proposed analytical method showed linear response for both gliclazide and metformin in the concentration ranges 1.25-150 µg/ml and 2.5-150 µg/ml respectively.



Linearity curve of metformin

- Gliclazide and metformin were well separated and repeatedly retained at the same retention times for triplicate determinations, where RSD% was less than 0.5% for all chromatographic parameters in terms of tailing factor, repeatability, number of theoretical plates and resolution between gliclazide and metformin peaks.

System suitability results, acceptance limit RSD% < 2

- Accuracy of the analytical method was expressed in terms of % recovery of gliclazide and metformin from spiked placebo matrix at 3 different levels (80%, 100% and 120%). Both analytes showed more than **99%** recovery to reflect the high accuracy of the proposed method.
- The method was sensitive enough to detect as low as **0.8** µg/ml of both gliclazide and metformin and to quantify as low as **2.4** µg/ml of both antidiabetic agents.
- The chromatographic method is specific for determination of both gliclazide and metformin in the presence of common tablet excipients.

- The peak areas obtained following triplicate analysis of the binary mixture were repeatable and precise over 3 different days. RSD% never exceeded 0.8% in all determinations.
- Intra and inter-day analytical results emphasized high system and method precision in determination of gliclazide and metformin.

Intra and inter-day precision results as peak area of different determinations on 3 consecutive days, (gliclazide 30µg/ml, metformin 30µg/ml) (n=3), acceptance limit RSD% < 2)

- The analytical method is robust to minor deliberate chromatographic variations such as flow rate, buffer pH, mobile phase composition, where no significant changes to the resolution between gliclazide and metformin peaks or to the sharpness of the separated peaks were observed.
- The method was successfully applied in the analysis of gliclazide and metformin in different pharmaceutically marketed products.

Conclusion

- The proposed validated method is simple, economic, rapid, sensitive, specific, accurate, linear and robust.
- The chromatographic method could be industrially applied in routine quality control analysis of gliclazide and metformin either in bulk or in pharmaceutically formulated products.
- The analytical method can be used for analysis of gliclazide alone, metformin alone or in combination.

Acknowledgements

The work is partially supported by the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 690676.

Appendix B: Author contribution to publications

I, Ahmed Gedawy, as the first author of the publication entitled " **Role of metformin in various pathologies: state-of-the-art microcapsules for improving its pharmacokinetics** ", declare that this work was primarily designed, interpreted, and written by the first author of this manuscript.

[redacted]
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I, as a co-author, endorse that this level of contribution by the first author indicated above is appropriate.

Hani Al-Salami
Co-Author 2 printed name

[redacted]
Co-Author 2 signature

Crispin R Dass
Co-Author 3 printed name

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ure

I, Ahmed Gedawy, as the first author of the publication entitled " **Polydimethylsiloxane-customized nanoplatform for delivery of antidiabetic drugs** ", declare that this work was primarily designed, experimentally executed, interpreted, and written by the first author of this manuscript.

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Crispin R Dass

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I, Ahmed Gedawy, as the first author of the publication entitled " **Development and validation of a new analytical HPLC method for simultaneous determination of the antidiabetic drugs, metformin and gliclazide** ", declare that this work was primarily designed, experimentally executed, interpreted, and written by the first author of this manuscript.

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Hani Al-Salami

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Crispin R Dass

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I, Ahmed Gedawy, as the first author of the publication entitled " **Advanced and multifaceted stability profiling of the first-line antidiabetic drugs metformin, gliclazide and glipizide under various controlled stress conditions** ", declare that this work was primarily designed, experimentally executed, interpreted, and written by the first author of this manuscript.

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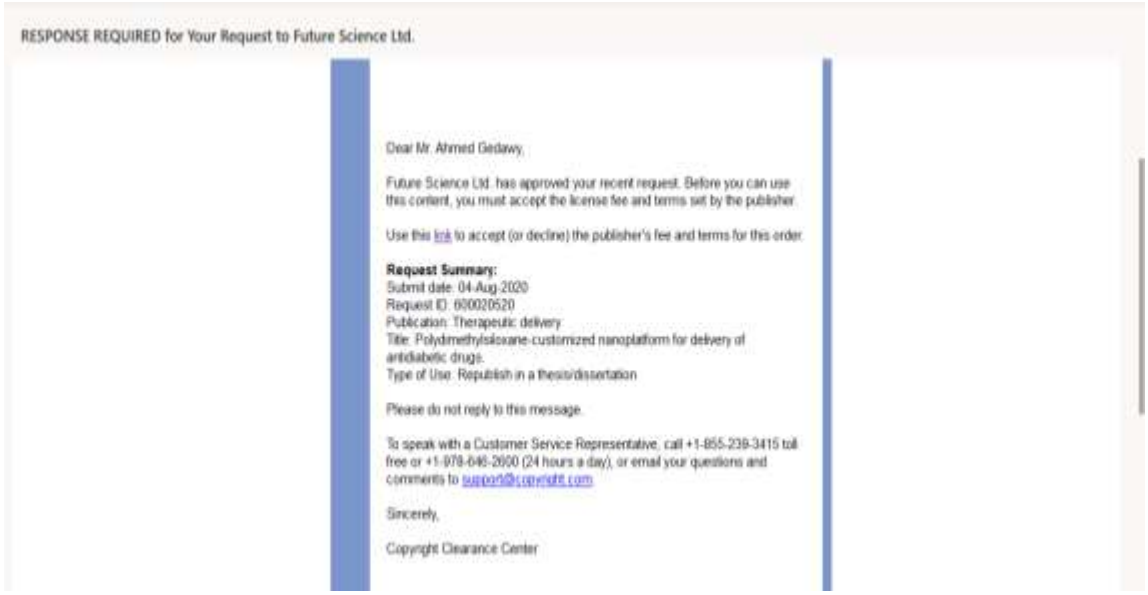

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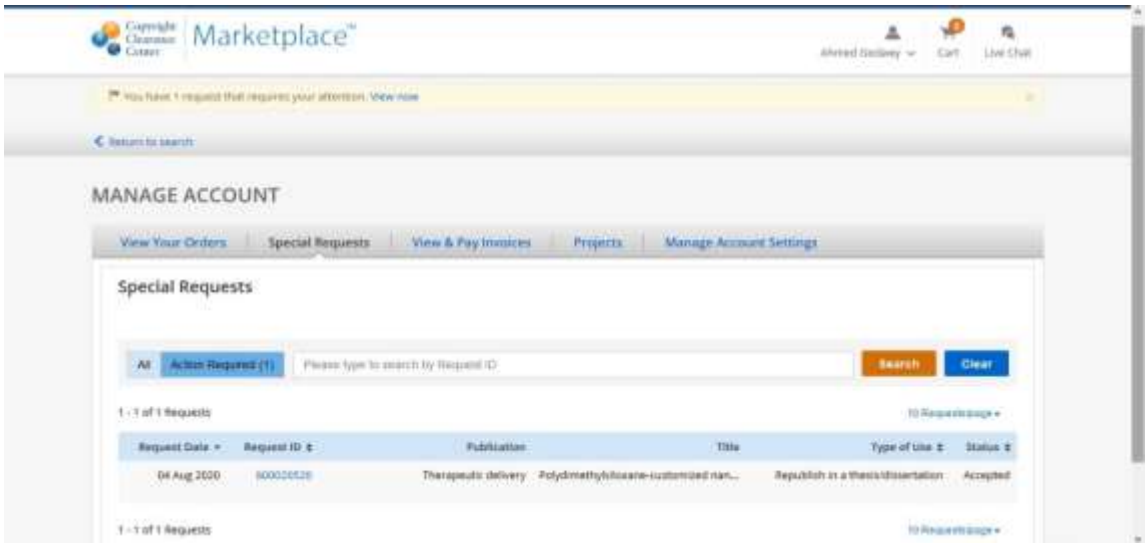

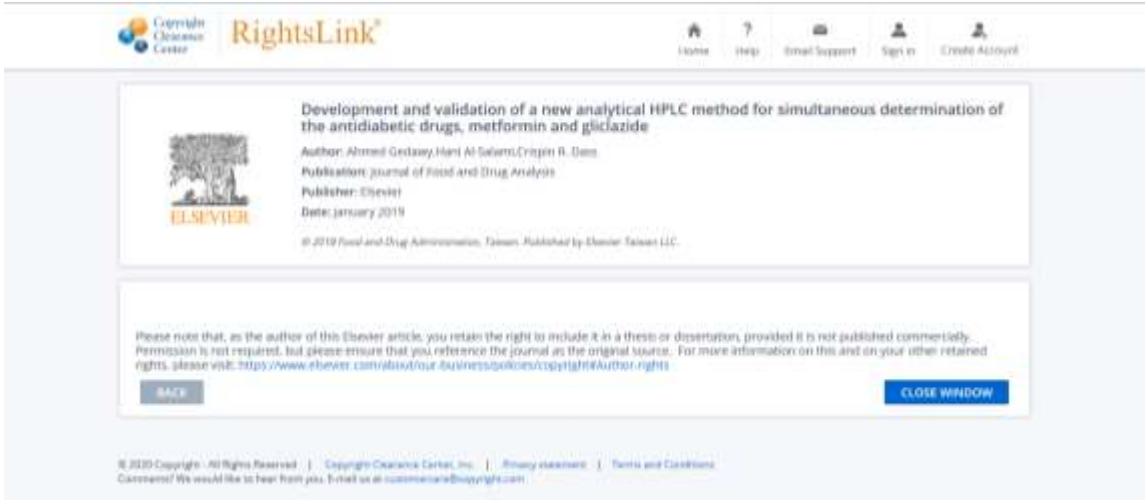
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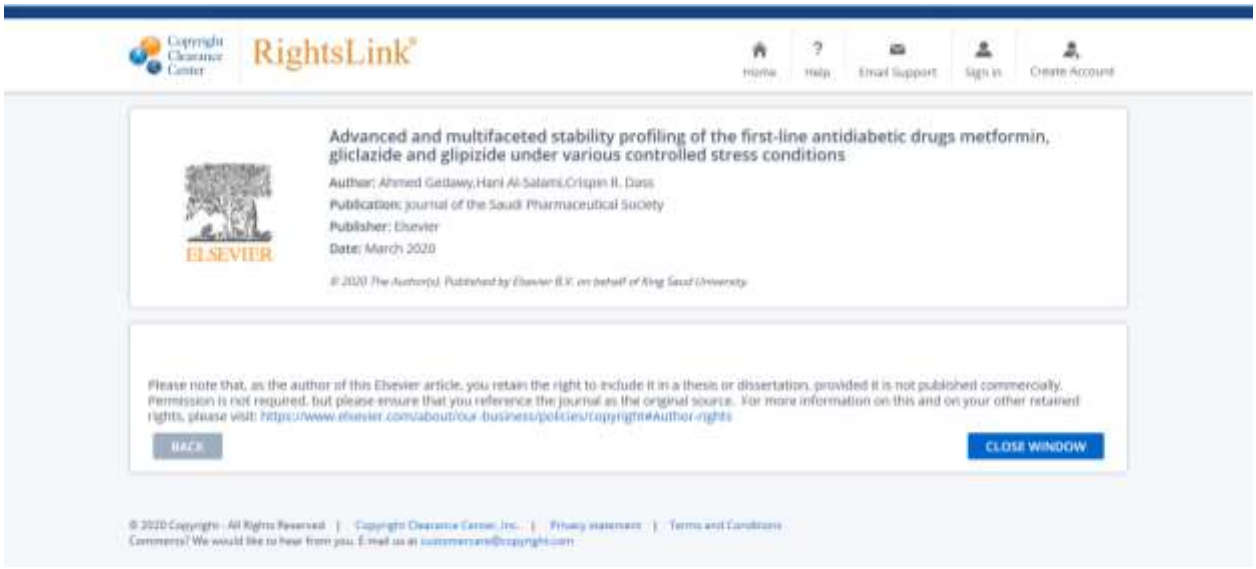
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