Title: A methodological evaluation and predictive *in silico* investigation into the multi-functionality of arginine in directly compressed tablets

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ABSTRACT

The acceleration of solid dosage form product development can be facilitated by the inclusion of excipients that exhibit poly-/ multi-functionality with reduction of the time invested in multiple excipient optimizations. Because active pharmaceutical ingredients (APIs) and tablet excipients present diverse densification behaviours upon compaction, the involvement of these different powders during compaction makes the compaction process very complicated. The aim of this study was to assess the macrometric characteristics and distribution of surface charges of two powders: indomethacin (IND) and arginine (ARG); and evaluate their impact on the densification properties of the two powders. Response surface modelling (RSM) was employed to predict the effect of two independent variables; Compression pressure (F) and ARG percentage (R) in binary mixtures on the properties of resultant tablets. The study looked at three responses namely; porosity (P), tensile strength (S) and disintegration time (T). Micrometric studies showed that IND had a higher charge density (net charge to mass ratio) when compared to ARG, nonetheless, ARG demonstrated good compaction properties with high plasticity (Y= 28.01 MPa). Therefore, ARG as filler to IND tablets was associated with better mechanical properties of the tablets (tablet tensile strength (σ) increased from 0.2±0.05 N/mm^2 to 2.85±0.36 N/mm^2 upon adding ARG at molar ratio of 8:1 to IND. Moreover, Tablets' disintegration time was shortened to reach few seconds in some of the formulations. RSM revealed tablet porosity to be affected by both compression pressure and ARG ratio for IND/ARG physical mixtures (PMs). Conversely, the tensile strength (σ) and disintegration time (T) for the PMs were influenced by the compression pressure, ARG ratio and their interactive term (FR); and a strong correlation was observed between the experimental results and the predicted data for tablet porosity. This work provides clear evidence of the multi-functionality of ARG as filler, binder and disintegrant for directly compressed tablets.

Keywords: Indomethacin, arginine, compressibility, compactibility, tabletability, disintegration time, multifunctional excipient, direct compression.

Abbreviation list

ANOVA	Analysis of variance
Arginine	ARG
APIs	Active pharmaceutical agents
CMR	charge–to–mass ratio
Copt	optical concentration
DCPD	dicalcium phosphate dihydrate
DoE	Design of experiment
Fc	Crushing force
g	Gram
IND	Indomethacin
Ln	Natural log
mg	Milligram
min	Minutes
MLR	Multiple Linear Regression
MPa	Mega Pascal
N	Newton
NSAID	Non-Steroidal Anti-Inflammatory Drug
pK_a	Acid dissociation constant
PM	Physical mixture
PSDs	Particle Size Distributions
P_Y	Yield pressure
SEM	Scanning Electron Microscopy
VMD	Volume median diameter
Y	Yield strength
ε	Porosity
Θ	Angle of repose
µg	Microgram
ρ_d	Bulk density
ρ_t	True density
σ_o	tensile strength at zero porosity

1.1 INTRODUCTION

Tablets are the most widely used dosage form nowadays. Many factors are involved during tablet manufacture in order to improve the quality of the final product; thus compaction of all ingredients into one tablet is a complex process [1]. The main reason behind such complexity is the contribution of the physical characteristics of each pharmaceutical powder during the compaction process [2, 3]. Therefore, a lot of scientific research is devoted to studying the compaction/densification behavior of individual powders.

The compaction behavior of binary mixtures has been investigated by many groups [1-8]. However, no simple relationships were observed between the properties of tablets prepared from binary mixtures and tablets prepared from single components. At least, three relationships have been discussed in literature: (i) linear (with both factors increasing/decreasing at the same rate), (ii) positive (both variables increase/ decrease simultaneously) and (iii) negative relationships (as one variable increases, the other decreases). The study carried out by Newton et al [9] found that tablets prepared from binary mixtures of dicalcium phosphate dihydrate (DCPD) and phenacetin had higher tensile strength than tablets prepared from either powder alone. The study suggested that this positive relationship was probably because the bonds formed between DCPD-phenacetin particles were stronger than the bonds within individual materials [9]. Similar results were reported later by Cook and Summers [10] when DCPD was co-compressed with aspirin. Although porosity, punch force and elastic recovery showed linear relationships with DCPD to aspirin composition, the tensile strength of the prepared tablets showed a positive relationship. Linear relationships have been reported by [9, 10] for different types of lactose. Such good correlations were attributed to the similarity in the compaction properties and the bonding mechanisms of the two components used in the study. On the other hand, mixtures of brittle lactose and plastic sodium chloride had a negative relationship. This was attributed to the ability of lactose to

undergo brittle fracture resulting in higher proportion of lactose-lactose bonding which is believed to be weaker than that of sodium chloride [1]. In general, it has been documented that tablets with increased tensile strength could be prepared by mixing a plastically deforming solid (e.g. Avicel®) with a fragmenting solid (e.g. paracetamol).

Despite the large number of studies conducted on binary mixtures, only few studies have managed to propose a model to predict the mechanical properties of compacted mixtures [11,12]. The percolation theory has been widely used to explain the change in properties exhibited by binary mixtures. For instance, in a binary mixture of materials A and B: if powder B exists in a small fraction it would form isolated inclusions and become the dispersed phase; while the powder A would form the continuous phase. The localized tension caused by the presence of a small amount of B, would result in a change in the behavior of powder A. Increasing the amount of B in the mixture would result in aggregate formation; and at a certain concentration of B, a network of B particles appears and this concentration represents the percolation threshold. Furthermore, during the compaction of A and B, three kinds of interactions can occur: (i) preferential bonding of A with A (A-A) and preferential bonding of B with B (B-B); or (ii) the affinity of A-A or B-B bonding is similar to the affinity of A-B bonding; or (iii) A bonds preferentially with B (A-B).

Indomethacin (IND) is a non-steroidal anti-inflammatory drug (NSAID) which has widely been used to reduce pain associated with rheumatoid arthritis, osteoarthritis, bursitis and headache [13]. A major challenge with IND is its low aqueous solubility. The compaction properties of IND solid dispersion with crospovidone was evaluated by Shibata [14], and the study found that better tablets were obtained using microcrystalline cellulose (Avicel®) and magnesium stearate and this was ascribed to the ability of Avicel® to minimize the ejection force and in turn reduce the adhesive tendency of the tablets. On the other hand, studies conducted by Tahvanainena *et al* [15] found that IND formed good tablets upon loading with thermally oxidized mesoporous silicone

microparticles. These tablets were found to have higher dissolution and permeability profiles than the free IND. Although many studies have evaluated the compaction properties of formulations containing IND, none of them investigated the densification mechanism of this anionic drug alone. Our previous salt formation studies have shown a 10^4-fold increase in IND solubility [16] and higher uptake by active carriers [17] in the presence of the ARG counter ion. Therefore, adding excess of the amino acid counter ion would act as a solubility and dissolution enhancer concurrently with bulking up the tablet. Thus, the overall aim of this work was to test the hypothesis that employing ARG as a filler in the tablet mix could provide an excess of ARG around IND molecules that could facilitate disintegration, solubility, dissolution and permeability. The objectives of the current study therefore included the characterization of compaction properties of two powders, namely IND as a model drug, and ARG as a tablet filler for the free drug. Powder flowability, particle size and surface charge distributions were first evaluated to help understanding the compaction profiles of these two powders. Thereafter, response surface modeling (RSM) was utilized to predict the compaction and disintegration characteristics of binary mixtures between the free drug and ARG (PMs) and compare the predicted results against the experimental data.

1.2. MATERIALS AND METHODS

1.2.1. Materials

Indomethacin (TLC ≥ 99%), L-ARG (non-animal source) and magnesium stearate were purchased from Sigma Aldrich (Dorset, UK). Ethanol was purchased from Fisher Scientific (Loughborough, U.K.)

1.2.2. Methods

1.2.2.1. Preparation of binary mixtures

Physical mixtures (PMs) of 20 grams made of IND with various molar ratios of ARG were prepared by weighing accurate amounts of IND and ARG followed by 10 minutes mixing using (WAB Turbula®,willy A, Bachofen AG, Switzerland). IND:ARG at molar ratios of 1:1, 1:2, 1:4 & 1:8 were prepared for further investigations in this study. All powder samples were kept at room temperature under desiccation until used for experimental work.

Particle size analysis

Volume-weighted particle size analysis of all individual powders and PMs was conducted using a Sympatec HELOS/RODOS (Clausthal-Zellerfeld, Germany) laser diffraction particle size analyser. The dispersion of air pressure was adjusted to 2.0-bar and a feed rate of 20% was applied. The particle size distributions (PSDs), i.e., particle size at 10% ($D_{10\%}$), 50% ($D_{50\%}$, median diameter), 90% ($D_{90\%}$) of the volume distribution and volume mean diameter (VMD) (mean ± SD, n = 3), were all calculated automatically using the WINDOX software based on Fraunhofer theory. Approximately 1 g of each powder was hand-fed into the VIBRI RODOS disperser through a funnel placed above the u–shaped groove of the rotating table. The sample container was cautiously tapped against the funnel to make sure the material was flowing through the vibrating chute into the groove of the rotary table. A background measurement was taken as the reference test. The measurements were set to trigger when the optical concentration (Copt) was higher than 1.1% and to end when the Copt fell below 1% for 5 s. The timebase was 100 ms and the obstruction was ~ 10-30%.

Powder flow properties

Powder flow was studied using an Erweka Granulate Flow tester (GTL type, Heusenstamm, Germany) and was determined from the flow time. About 10 g of each sample was poured

through 6.0 mm nozzle coupled to the equipment. The time taken to discharge each powder was recorded. The results were taken as the average of 6 replicates (n = 6).

Angle of repose (Θ) is the most commonly used method to describe powder flowability (Kumar et al., 2004). In order to calculate Θ, a pile was built by dropping 10 g of powder through a 75 mm diameter glass funnel on a flat surface. The height between the base where the powder was poured and the funnel tip was 5 cm. The angle of repose (Θ) was then calculated by the following equation:

$$Tan\ \theta = \frac{2h}{D} \quad \text{Equation 1}$$

where h is the height of the powder cone and D the diameter of the base of the formed powder pile.

1.2.2.3 Scanning Electron Microscopy (SEM)

Zeiss scanning electron microscope (Evo50, Oxford instruments, Inca wave, UK) was used to study the surface morphology and particle size of IND and ARG powders. Fine powder samples were lightly sprinkled on the carbon surfaces of universal specimen stubs and double coated with gold under low vacuum for about 4 minutes in the presence of Argon gas, using a sputter coater (Polaron SC500, Polaron Equipment, Watford, UK) at 20 mA. The particle surface morphology was captured and analysed using smartSEM software.

1.2.2.4 Evaluation of electrostatic charge properties

A tribo–electric device electrostatic inductive sensor was used to investigate the tribo–electrification of formulation powders under investigation. A sample of each powder was fed in the cylindrical sensor with the help of vibratory feeder and conveyed toward the sensor by gravity in a vertical direction. Special care was taken by considering the adhesion property of particles

with the wall of the sensor. After each experiment, the inner tube was replaced in order to remove any deposits, impurities or surface charge that may have been present on the surface from a previous test. A fresh sample was used for each test experiment. Each sample was analysed four times under humidity and temperature controlled laboratory: 50% RH, 22 ^{o}C. The positive charge is the sum of positive charges whereas the negative charge is the sum of negative charges. The net charge is the sum of positive charges and negative charges, whereas the overall absolute charge is the sum of absolute charges detected regardless of polarity. The charge–to–mass ratio (CMR or charge density) is defined as the charge (negative charge for N–CMR, positive charge for P–CMR, net charge for net–CMR, or overall absolute charge for absolute–CMR) per unit mass, in nC/g.

1.2.2.5. Tablet preparation

In order to evaluate the compressibility and tabletability of IND, ARG and their binary mixtures, 500 mg of the powders were accurately weighted out and directly compressed. The powders were directly compressed using a uniaxial hydraulic press (Specac tablet presser, Kent, UK) and 13 mm split die which prevents mechanical failure by allowing triaxial decompression. Magnesium stearate in ethanol (5% w/v) was used to lubricate the die walls and punch surfaces. Powders were compressed at 5, 10, 20, 30, 40 and 60 kN, (37.7, 75, 150, 226, 302 and 452 MPa) with dwell time of 30 sec. Cylindrical tablets with diameter of ~13 mm and flat faced surface were obtained. Tablets were left in desiccators until performing any mechanical testing.

1.2.2.6. Tablets' porosity measurements

Bulk densities of the prepared tablets were determined by using digital calliper by measuring the dimensions pre-weighed tablets. Apparent tablets densities were measured using a helium

pycnometer (Multipycnometer Quantachrome Instruments, Hampshire, UK). Both bulk and true density values were used to determine the tablets' porosity using equation 2.

$$\varepsilon = 100(1 - \rho_d / \rho_t)$$ **Equation 2**

Where ε is the porosity and ρ_d is the bulk density and ρ_t is the true density. All the measurements were done in triplicate.

1.2.2.7. Tablet tensile strength measurement

The force required to crush the tablets was measured using a tablet hardness apparatus (Schleuniger 4M, Thun, Switzerland). The measured force was used to determine the tablet tensile strength using equation 3.

$$\sigma = \frac{2F_c}{\pi dt}$$ **Equation 3**

Where σ is the tablet tensile strength, F_c is the crushing force required to break the tablet, *d* is the tablet diameter and *t* is the tablet thickness. All measurements were done in triplicate.

Tensile strength at zero porosity was calculated using Ryshkewitch equation (equation 4).

$$\sigma = \sigma_0 e^{-b\varepsilon}$$ **Equation 4**

Where σ is the tensile strength, σ_0 is the tensile strength at zero porosity and is widely used to measure the inherent bonding propensity of a powder, ε is the porosity and *b* is an empirical constant.

1.2.2.8. Heckel analysis

The Heckel equation (5) was used to analyse the tablet's compression characteristics [18,19].

$$ln\left(\frac{1}{1-D}\right) = KP + A$$ **Equation 5**

Where D is the tablet relative density at pressure P, K is a material constant (slope of the straight line portion of the Heckel plot) and is 1/3 of the yield strength. The 1/K value is used to express

the mean yield pressure and gives an indication of the material's ability to undergo plastic deformation under pressure. A is a function of the initial bulk volume and is calculated from the intercept of the straight line of the Heckel plot. It provides information on the movement/rearrangement of particles at the initial stages of compaction.

1.2.2.9. Disintegration time studies

In vitro disintegration time was evaluated using the US pharmacopoeia monograph (<701> disintegration). An Erweka ZT3, GMBH (Heusenstamm, Germany) was used in this study as the disintegration apparatus and distilled water (800 ml) as disintegration medium; temperature was thermostatically maintained at 37 °C. Three tablets were placed in the basket rack assembly and covered with a transparent plastic disk. The disintegration time was taken as the time required for tablets to disintegrate completely without leaving any bulk solid residue. All the measurements were carried out in triplicate and presented as mean ± standard deviation.

1.2.2.10. Design of experiment (DoE)

MODDE software version 8 (Umetrics inc., CA, USA) was used to design the statistical experiment for this study. A three-level full factorial response surface modelling (RSM) design with 24 total runs including three replicated centre points was selected. The effects of two independent variables (factors): compression pressure (F) and ARG ratio (R) on three dependant variables (responses): porosity (P), tensile strength (S) and disintegration time (T) were studied. RSM was preferred over screening modelling because the aim of the study was to predict effects of both compression pressure and ARG ratio on tablets characteristics. A full factorial design with each factor varied at three levels was selected; and the model was fitted to multiple linear regression (MLR) because the total number of responses was not more than three [33]. The significance of the quadratic model (linear, interactive and polynomial) on the dependent variables was carried

out using analysis of variance (ANOVA) and the quantitative effects of the independent variables on the responses was considered significant at a 95% confidence level ($p < 0.05$).

1.3. RESULTS AND DISCUSSION

1.3.1. Powder Characterisation

Characterization of particle size, charge and morphology

Despite the wide research on the applications of arginine in food [21, 22] and pharmaceuticals industries [23], the compaction properties of this amino acid were never investigated. Prior to evaluating the compaction behaviour of ARG, the micromeritic characteristics; particle size, surface charge and surface morphology were first evaluated. Particle size distribution ($D_{10\%}$, $D_{50\%}$, $D_{90\%}$ and VMD) of ARG, IND and IND:ARG physical mixtures at different ratios (1:1, 1:2, 1:4 and 1:8) are shown in Fig. 1. DLS demonstrated that ARG has considerably larger particle size compared to IND, (e.g. VMD: 131.0 ± 10.4 µm versus 46.6 ± 0.5 µm) (Fig. 1), which is further supported by scanning electron microscopy (Fig. 2). Particle size distributions of IND:ARG physical mixtures varied considerably, with the VMD increased with increasing the IND:ARG ratio (Fig. 1). SEM images also demonstrated that IND crystals are irregular in shape with sharp edges with particle size greater than 20 µm. On the other hand, Arginine crystals had cubic shapes with rough surface and displayed a size of ~100 µm under the microscope (Fig. 2).

Electrostatic charge behaviour of different powders was also studied and showed significant variations (Fig. 3) between the individual powders and the physical mixtures. In comparison to ARG, indomethacin showed significantly higher charge density (positive charge–to–mass ratio (P–CMR), negative charge–to–mass ratio (N–CMR) and net charge–to–mass ratio (net–CMR)). This may, in part, be attributed to the small size of IND in comparison to ARG (Fig. 1) since it has been

reported that small particles may carry higher charge density than large particles [24]. It is believed that smaller particles have larger specific surface area which in turn increases the number of contacts between particles and surrounding surfaces. These findings were also discussed by [25]. Although surface roughness is believed to have an effect on the surface charge of the particles, it is envisaged that this effect is minimal in pharmaceutical powders as they have low surface roughness [26] (Eilbeck et al., 1999)

The highest net-CMR was observed for IND-ARG (1:2) whereas the lowest net-CMR was observed for IND-ARG (1:8).

Flowability studies

Upon investigating the flowability of various powders, IND powder showed the poorest flow behaviour amongst the other powders (flow time: 4.0 ± 0.1 s) (Fig. 4a). This may be related to the of IND particles having the smallest particle size among powders under investigation (Fig. 1). Plotting flow time against VMD of IND, IND:ARG PMs and ARG revealed a linear relationship (r^2 = 0.8617), suggesting that flow behaviour of the IND:ARG PM powder improves with increasing ARG ratio i.e. increasing the particle size (Fig. 4b). The flow properties for the powders were additionally evaluated using angle of repose (Θ) (Table 1). IND exhibited fair flowability (Θ= 36.89 ± 3.28°), whilst ARG had excellent flowability (Θ = 29.4 ± 0.84°). This may be ascribed to the smaller size (Figs. 1 and 2) and higher charge density (Fig. 3) of IND in comparison to ARG. The negative surface charge carried by IND (net-CMR: -9.35.1 pC/g) increases the adhesiveness of the powder to surfaces and in turn daunt the flow of the powder as suggested by [27].

Apart from IND alone powder, flow time and angle of repose were indirectly proportional (r^2 = 0.8258) (Fig. 4c) suggesting a reasonable agreement between the two methods applied in this study. In contrast to IND:ARG and ARG alone powders, angle of repose measuring of IND alone is less accurate due to its high cohesiveness, thus potential blockage during testing.

1.3.2. Densification properties of individual powders

The compaction properties of each individual powder were studied to understand their densification mechanisms prior to conducting the RSM studies for the binary mixtures. The reduction of the material volume that accompanies an applied pressure is known as compressibility [28]. The compressibility performance of pharmaceutical powders is evaluated by plotting tablet porosity against the compaction force/pressure [28]. For a pharmaceutical powder to form a strong compact, the material should be highly compressible; i.e. have smaller porosity upon compression in order to enable particles to get closer to each other and increase the bonding area between the particles [28]. Fig. 5 shows the compressibility profile of the two individual powders; IND and ARG. Generally, increasing the compression pressure was associated with a decrease in tablet porosity. IND showed lower compressibility profile than that of ARG (the tablet porosity was 0.52 at 37.7 MPa and only slightly dropped to 0.46 upon doubling the compression pressure, then no significant ($p>0.05$) change was observed even at the very high compression pressures.

It is known that the plastic properties of pharmaceutical powders play an important role during the densification of the powder. In order to understand the densification mechanism of the individual powders, Heckel analysis was carried out using out-of-die method. Yield strength and yield pressure were calculated using Heckel equation (equation 5) and summarised in Table 1. Heckel plots for the powders showed initial curvature representing the particle fragmentation and rearrangement in the die. This was followed by a linear relationship between compression pressure and -ln porosity, the slope of which indicates the degree of plastic deformation and consequently yield strength [20]. The yield strength of ARG was found to be 28.01, which reflects the high plasticity of the powders, as lower yield strengths (or yield pressures) imply higher plastic deformation [19, 20]. Nevertheless, the analysis showed a very low plastic characteristic and high

elastic deformation of IND during compaction as the yield strength was found to be 208.33. The high elastic behaviour of IND affected the integrity of its prepared tablets and problems such as capping, lamination and breakage were observed.

The ability of pharmaceutical powders to form a strong tablet during densification is known as compactibility and can be represented by plotting the tensile strength against porosity.

The compactibility profile of IND and ARG were studied and are summarised in Fig. 6.

As suggested by [29] an exponential correlation between tablet tensile strength and porosity was observed for ARG i.e. increasing the tablet porosity resulted in an exponential decrease in the tablets' tensile strength. In order to evaluate the inherent bonding propensity of ARG and IND, tablets' tensile strength at zero porosity (σ_0) was calculated using Ryshkewitch equation. The empirical constant (*b*) is related to pore distribution within a tablet and represents its bonding capacity. Higher values of *b* imply greater bonding capacities, since it indicates that tablet tensile strength increases exponentially, with decreasing tablet porosity [30].

σ_0 of ARG was found to be 381 demonstrating high compactibility of ARG which reflects the strength of bond formation, this suggests that strong bonds are formed during the densification of ARG. On the other hand, IND showed a weak correlation (Fig. 6) and low compactibility (σ_0= 1.13) indicating, the weak bond formation under compaction.

A third property is tabletability which is the ability of powder to form a tablet of specific strength while exposed to compression pressure [28]. The tabletability profile of a pharmaceutical powder can be expressed by plotting tablet tensile strength against compression pressure [30]. Both powder compressibility which affects the bonding area and compactibility properties of the powder which reflects the strength of bond formation, determine the tabletability characteristics of the pharmaceutical powder. Fig. 7 shows that increasing the compression pressure resulted in an increase in tensile strength of ARG tablets. ARG tablets showed a tensile strength of 0.04±0.02

N/mm^2 that increased steadily to 4.2±0.13 N/mm^2 at the highest compression pressure. On the other hand, IND powder tabletability was consistent regardless to the compression pressure applied. Maximum tensile strength of IND was (0.23 ± 0.05 N/mm^2) at compression pressure 75.4 MPa. This result was expected, as Heckel analysis revealed the high elasticity of IND while the compactibility data showed weak bond formation. On the other hand, the cationic amino acid tablets showed the highest tensile strength reflecting good tabletability properties.

1.3.3 Disintegration Studies

The ability of the tablets to disintegrate in water was evaluated. The disintegration results revealed that increasing the compression pressure increased the tablets' disintegration time. Although compressibility data revealed the high porosity of IND, the free acidic drug showed a very poor disintegration and the drug failed to disintegrate even after 20 minutes. The high lipophilicity of IND could be the reason behind its poor disintegration. Hansch and Leo [31] reported the log P value of IND to be 3.8 which reflects its low hydrophilicty, low wettability [32] and hence the extended disintegration time ; irrespective of its high tablet porosity. On the other hand, ARG tablets showed very rapid disintegration time (25.3±2 s) for tablets compressed at 226 MPa despite having high tensile strength (1.23±0.09 N/mm^2). This was probably because of the high hydrophilicity of ARG with low log P value of 0.69 [32] and therefore high wettability and fast disintegration.

1.3.4 DoE for comparison of predicted and experimental data for binary mixtures

1.3.4.1. Compressibility characterisation of the binary mixtures

The characterisation of the individual powders showed that ARG had very high bonding capacities and its tablets disintegrated quickly. Thus, incorporating this amino acid into formulations would

be expected to improve the tablets' characteristics. Moreover, a previous study conducted by [17] showed that adding ARG to anionic drugs improved drug dissolution and absorption through intestinal membranes [17]. Therefore, an experimental design was used to predict the effect of incorporating ARG to IND formulations, which showed very poor compaction and disintegration characteristics, in an attempt to derive a model that could predict the compaction properties of the binary mixtures and compare these against experimental data. Such a model would contribute significantly to predicting the compaction behaviour of binary mixtures whilst utilising fewer experimental runs and resources.

The compression force is an important factor that affects the porosity and tensile strength of the tablets. Hence the compression force (F) was selected as an independent variable and ranged from 5-60 kN equivalent to compression pressure of (37.7, 75, 150, 226, 302 and 452 MPa). The ARG ratio (R) –ranging from 0 to 100% in the binary mixture was the second variable and was selected for the reasons mentioned above. The effect of these two independent variables on tablet porosity (P), tensile strength (σ) and disintegration time (T) was studied using response surface modelling (RSM). RSM utilises mathematical and statistical techniques to develop, improve or optimise products [33]. Table 2 summarises the effect of each independent factor on the three responses. The sign of the effect represents the trend while the values indicate the magnitude of this effect. The data showed that both compression force (F) and ARG ratio (R) had a significant ($p< 0.001$) linear influence on IND tablet porosity; the quadratic term for compression force (F^2) also affect porosity significantly. As expected, Fig. (9A) shows that increasing the compression force and ARG ratio causes a decrease in the tablets porosity because high compression force bring particles into closer proximity with each other, thereby decreasing tablet porosity [1]. Nevertheless, the effect of compression force (0.16) was twice that of ARG ratio (0.08); thus compression force played a higher role in tablet porosity than amino acid ratio. Interestingly, the

interactive term FR was found to have an antagonistic effect as the magnitude of decrease in the tablet porosity was less than the effect of each single term. A closer look at Fig. 9A showed that at low compaction forces, increasing ARG ratio had no effect on tablet porosity; but at higher compaction forces (middle portion of the plot), an increase in ARG ratio increased tablet porosity; thus substantiating the effect for the interactive term.

MLR was used to fit the data and this generated mathematical equations which could be used to predict the tablet porosity by inputting the experimental data using the two independent variables - F and R. The mathematical expression for working out the porosity of IND/ARG binary mixtures is given in equation 6.

$$P = 0.343 - 0.159F - 0.078R + 0.15F^2 - 0.062FR$$ **Equation 6**

Where P is the porosity for IND/ARG PMs, F is the compression force and R is ARG percentage in the binary mixture.

The compressibility profiles for PMs were studied in order to compare the experimental findings against the predicted data. Fig. 10 shows the compressibility profile for all IND:ARG PMs.

In a similar way to individual powders, increasing the compression pressure resulted in decreasing tablet porosity. The highest compressibility was exhibited by PM 1:4> PM 1:2> PM 1:8 > PM 1:1. Heckel analysis (Table 1) showed that PM 1:4 had the highest plasticity as its yield strength was found to be (20.83) and the elasticity of IND (yield strength of 208.33) dropped significantly upon incorporating ARG into the formulations (yield strength of 61.7 at PM 1:1). These results confirmed the quadratic model findings which indicated that significant reduction in tablet porosity occurs upon addition of ARG.

1.3.4.2. Tabletability characterisation of binary mixtures

In order to predict the ability of ARG binary mixtures with IND to form a tablet of specific strength while exposed to different compression pressures (tabletability), RSM was carried out and Fig. (9B) was generated for IND/ARG PMs. The predicted data showed that tensile strength of IND tablets was significantly ($p< 0.001$) increased upon increasing both the compression pressure (F), ARG ratio (R) as well as their interactive term (FR). F and R were found to have roughly the same magnitude of effect on the tablets' tensile strength: 1.73 and 1.79 N/mm^2 respectively (Table 7). Interestingly, the interaction of both factors (FR) was found to have a synergistic effect (2.032) on tablet strength. This effect could be explained by the ability of ARG to improve the compressibility of IND tablets which in turn improves the inter-particulate bonding area [34]; besides its ability to enhance bond formation during densification as reflected by the compactibility studies. Because both compressibility and compactibility are pre-determinants of tablet strength, an increase in the tensile strength was observed. Moreover, this synergistic effect could reflect strong bonding between IND and ARG with highest hardness value at 50% ARG (PM 1:1); which also displayed very high σ_0 value; and was earlier suggested by Sheikh-Salem and Fell [2, 34]. None of the polynomial terms (F^2 or R^2) were found to influence ($p>0.05$) the tablets hardness.

Equation 7 was generated after the model was fitted to MLR, and could be used to predict the tablets tensile strength if the compression pressure and ARG ratio are known.

$$S = 1.022 + 1.73F + 1.79R + 2.03FR \quad \textbf{Equation 7}$$

Where S is the tensile strength for IND/ARG PMs, F is the compression force and R is the ARG percentage.

Experimental studies to verify the trends predicted by the RSM model for PMs were undertaken and results are summarised in Fig. 11.

Similar to the individual powders, a linear positive relationship was observed between tensile strength and compression pressure, i.e. increasing the compression pressure resulted in increasing

tensile strength for all the formulations. The highest tabletability was shown by PM 1:2 ≈ PM 1:4> PM1:8 >PM 1:1 (Fig. 10). The similar tabletability of PMs 1:2 and 1:4 may be due to compressibility and compactibility similarities between them (as discussed earlier).

1.3.4.3. Disintegration studies for binary mixtures

Disintegration time was the last response evaluated in this study. The significant terms affecting disintegration time for IND/ARG PM tablets were identified as compression pressure (F), ARG ratio (R), the interactive term (FR) and the polynomial/ quadratic term of ARG ratio (R^2). Increasing the compression force (F) was found to increase the disintegration time by a magnitude of (262. 2), while increasing the ratio of ARG in the mixtures was found to decrease the time required for the tablets to disintegrate significantly ($p< 0.001$) and even by higher magnitude (1069.5) when compared to the compression pressure (F) (Table 2). This portrays the disintegrant characteristic of ARG. Although the ARG ratio had a higher linear effect on the disintegration time than the compression pressure, the interactive term (FR) was found to delay the tablets disintegration for the PMs (Table 2).

Equation 8 can be used to describe the effect of the two independent factors: compression force and ARG ratio on the effect of disintegration time for PMs.

$$T = 556.055 + 262.16F - 1069.51R + 250.25R^2 + 170FR \qquad \textbf{Equation 8}$$

Where T is the disintegration time for IND/ARG PMs, F is the compression force and R is the ARG ratio. The three dimensional surface plots were generated using the software and depicts the effect of compression pressure and ARG ratio, individually and simultaneously, on the disintegration time for the PM (Fig. 9C).

The experimental data for the effect of compression pressure and ARG ratio on the disintegration time for the PMs are summarised in Fig. 12. The addition of ARG to IND tablets was found to reduce the disintegration time significantly (Fig. 12). It usually takes > 20 minutes for IND tablets (compressed at 37.7 MPa, 5 kN) to disintegrate, but upon adding 50% ARG (PM 1:1) to the formulation, the disintegration time was reduced to 195 ± 117 s. This decrease in disintegration time continued with increasing ARG ratio. The positive effect of compression force (F) on the disintegration time was significant at low pressures and up to 226 MPa (30 kN); beyond which the effect of force was insignificant. This data was in line with the RSM findings which suggested that the ARG percentage is the major determinant of tablet disintegration time (Table 2).

The correlation between the predicted values and the actual values of tablets' porosity, tensile strength and disintegration times were computed (Fig. 13). A fair correlation (R^2= 0.89) was observed between the predicted and actual values for tablets porosity; while weaker correlation was observed for that of tablets' tensile strength and disintegration time R^2= 0.77 and 0.61 respectively. Disintegration time showed the weakest correlation and this may be because the test is highly subjective, and depends on the evaluation of the experimenter, unlike the other tests that are more machine-dependent [35].

1.4. CONCLUSION

Individual powder characterisation showed good flow properties for ARG because of the large particle size and low charge density. Moreover, tablet characterisation showed that ARG had good compressibility, compactibility and faster disintegration time. Heckel analysis revealed high plasticity (Y= 28.01 MPa) of ARG and this explains the good mechanical properties of the prepared tablets with high ARG ratios. RSM revealed that both compression force and ARG ratio had a

significant impact on tablet porosity, tensile strength and disintegration time. Increasing compression pressure significantly decreased porosity, increased tensile strength and increased disintegration time of ARG and IND binary mixtures, while increasing ARG ratio significantly increased tensile strength and decreased disintegration time. Thus, the good micrometric properties and multi-functionality of the amino acid would enable its use as a bulking agent in manufacturing directly compressed tablets.

Compliance with Ethical Standards

Disclosure of potential conflicts of interest

The authors declare no conflict of interest.

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