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Research paper

# Formulation and characterisation of lyophilised rapid disintegrating tablets using amino acids as matrix forming agents

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ABSTRACT

Despite recent advances in the formulation of lyophilised rapid disintegrating tablets (RDTs), the inclusion of matrix supporting/disintegration enhancing agents has been limited to the use of saccharides and polyols. In this study, the feasibility of using amino acids as matrix forming agents in lyophilised RDTs was investigated. Twelve amino acids were chosen (alanine, arginine, threonine, glycine, cysteine, serine, histidine, lysine, valine, asparagine, glutamine and proline), and the suitability for freeze drying, mechanical properties and disintegration time after inclusion of the amino acids at varied concentration were studied. In addition, the porosity of the RDTs and wettability profile of the amino acids were investigated to understand the mechanisms of disintegration. The results suggest the suitability of these amino acids for the lyophilisation regime, as they displayed satisfactory safety margin between the glass transition and shelf temperature (−40 °C), except proline-based formulations. Moreover, the crystallisation behavior of alanine, glycine, cysteine and serine at high concentration increased the stability of the formulation. The characterisation of the RDTs suggests that high concentration of the amino acids is required to enhance the mechanical properties, whereas only optimum concentrations promote the disintegration. Moreover, wetting time of the amino acid and porosity of the tablet are the two factors that control the disintegration of RDTs.



## 1. Introduction

Over the last few years, a great deal of interest has been directed towards formulating solid oral dosage forms that disintegrate/dissolve rapidly in the mouth without the need for water. These dosage forms, known as rapid disintegrating or fast melt tablets, were primarily designed to offer a practical solution for patients who suffered from difficulty in swallowing conventional tablets and capsules. Moreover, besides improving patient compliance, rapid disintegrating tablets (RDTs) carry a plethora of advantages such as rapid onset of action [1], enhancement of patent life cycle management [2] and increasing the bioavailability of poorly water soluble drugs [3].

Currently, several approaches are widely used to fabricate RDT including lyophilisation, direct compression and moulding. However, in terms of sales value, sales volume and number of products available on the market, lyophilisation (freeze drying) method has been the most successful [4]. The fabrication of lyophilised RDTs is based on creating a porous matrix by subliming the water from pre-frozen aqueous formulation of the drug containing matrix forming agents and other excipients such as lyoprotectants, preservatives and flavours [1]. The matrix of the lyophilised RDT consists of two components that work together to ensure the development of a successful formulation. The first component is water soluble polymers such as gelatin, dextran, alginate [1] and maltodextrin [3]. This component maintains the shape and provides mechanical strength to the tablets (binder). The second constituent is matrix supporting/disintegration enhancing agents such as sucrose and mannitol, which acts by cementing the porous framework provided by the water soluble polymer and accelerates the disintegration of the RDT [5]. Although there is wide availability of literature describing the preparation of RDTs by lyophilisation, the number of matrix supporting/disintegration enhancing agents used has been limited to saccharides and polyols with majority of the work dedicated to the inclusion of mannitol [1,5]. This is primarily because the incorporation of these matrix forming agents requires fulfillment of stringent characteristics such as reasonable drying time, stability during freeze-drying process, as well as formation of elegant tablets with short disintegration time and adequate mechanical properties. However, high concentration of saccharides and polyols is required to achieve these quality features [1,5] thus restrains their application in delivering drugs for the treatment of long-term chronic conditions especially for children, diabetic and obese patients, due to limited intake requirement. Therefore, this

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current study aims to explore alternative novel excipients by investigating the feasibility of using amino acids as matrix supporting agents (second component) in the fabrication of rapid disintegrating tablets prepared by freeze drying in order to produce tablets with enhanced properties and wider application to pediatric and geriatric patient population.

Amino acids are the basic structural units (monomer) of proteins. An alpha amino acid consists of an amino group, a carboxyl group, a hydrogen atom and a distinctive side chain bonded to a carbon atom (alpha carbon). Basically, the side chains of amino acids are responsible for the variation in their physicochemical properties. Naturally occurring amino acids can exist in both the L (laevo) and the D (dextro) forms, which are mirror images of each other. However, incorporation of the D form of the amino acid has been limited for pharmaceutical applications due to their potential pharmacological activity, microbiological concerns and toxicity [6–8]. On the other hand, the L form of the amino acids has been used extensively in pharmaceutical and cosmetic formulations such as pH-sensitive drug carrier [9], cicatrisation topical dermatological preparations [10], salt conjugate of poorly soluble drug [11], oral tablets, as lubricant [12] and disintegration enhancer [13], inhalable delivery systems [14] and freeze-dried product, as cryoprotectants [15] and bulking agent [16].

In this study, L-amino acids with adequate aqueous solubility, which allow their inclusion at varied concentration, were chosen (alanine, arginine, threonine, glycine, cysteine, serine, histidine, lysine, valine, asparagine, glutamine and proline), and their potential as matrix supporting/disintegration enhancing agents was investigated individually at concentration of 10%, 30%, 50% and 70% w/w (total solid) using 5% aqueous solution of low bloom strength gelatin (60 bloom strength) as a binder. The formulations were examined for their thermal properties in their frozen state in order to explain their behavior during the freeze-drying process. The freeze-dried tablets were evaluated for their disintegration time and mechanical properties. In addition, the porosity of the RDTs and the wettability profile of the amino acids were investigated to explain the disintegration time and mechanism.

## 2. Materials and methods

### 2.1. Materials

Gelatin of bloom strength 60, L-alanine, L-arginine, L-threonine, glycine, L-cysteine, L-serine, L-histidine, L-lysine, L-valine, L-asparagine, L-glutamine and L-proline were purchased form Sigma–Aldrich Chemicals (Pool, UK). All the chemicals were of analytical grade.

### 2.2. Methods

#### 2.2.1. Preparation of lyophilised tablets

The amino acids were added individually to 5% (w/w) gelatin (60 bloom strength) stock solutions at concentrations of 10%, 30%, 50% and 70% of total solid material. From the stock solutions, 1.5 g was poured into the tablet mould (13.80 mm diameter, 8.50 mm height), frozen at −80 °C for about 60 min and freeze-dried (ADVANTAGE Freeze-dryer, VIRTIS) according to an optimised regime (primary drying for 48 h at shelf temperature of −40 °C and secondary drying for 10 h at shelf temperature of 20 °C and vacuum of 50 mtorr). All formulations were prepared in triplicate from three independent batches.

#### 2.2.2. Differential scanning calorimetry studies

Differential scanning calorimetry (Pyris Diamond DSC and Intracooler 2P: Perkin Elmer, Wellessey, USA) was used to determine the glass transition temperature ($T_g$) and crystallisation event of the formulation in its frozen state (before freeze drying). From the liquid formulation, 10–15 mg of the was loaded into aluminium pans, cooled to −65 °C and then heated to 20 °C at 5 °C/min with a nitrogen purge of 20 ml/min. To determine the glass transition temperature of the maximally freeze concentrate sample ($T'_g$), after initial cooling to −65 °C, annealing for 10 min at −15 °C, it was added before carrying out the above method. An empty aluminium pan was used as reference for all measurements.

The resulting plots were analysed by Pyris manager software. $T_g$ and $T'_g$ values were determined from the intersection of relative tangents to the baseline. All the measurements were taken in triplicate from independently prepared samples.

The DSC was calibrated for temperature and heat flow using standard samples of indium (melting point: 156.6 °C, ΔHm: 28.42 J/g) and Zinc (melting point: 419.5 °C, ΔHm: 108.26 J/g).

#### 2.2.3. Mechanical properties of the tablets

The mechanical properties of the tablets (hardness) were investigated with a texture analyzer (QTS 25: Brookfield, Essex, UK) equipped with a 25-kg load cell. The instrument was calibrated with standard weight of 500 g and 5 kg. The tablet was placed in a holder with a cylindrical hole. The hardness was taken as the peak force after 1-mm penetration of 5-mm-diameter probe at a speed of 6 mm/min. The results were average of three measurements from independently prepared batches.

#### 2.2.4. Disintegration time of the tablets

The disintegration time of the tablets was measured using a USP disintegration tester (Erweka, ZT3). Distilled water (800 ml) kept at 37 °C was used as a medium, and the basket was raised and lowered at a fixed frequency of 30 cycles/min. One tablet was tested at a time. All the formulations were evaluated in triplicate, and standard deviation was calculated.

#### 2.2.5. Porosity

The relative porosity was calculated from the apparent and strut density of the tablet. Apparent density was found by dividing the mass of the tablet by the measured volume. The strut density was determined using helium pycnometry (Accupyc 1330, Micromeritics, UK) with 3-$cm^3$ sample cup at 22 °C. Prior to analysis, the helium pycnometry was calibrated against a standard steel ball. Each determination included 10 purges at 19.5 psi and 10 analytical runs at 19.5 psi with an equilibration rate of 0.0050 psi/min.

#### 2.2.6. Wetting profile

The wetting profile of the amino acids was analysed by measuring their contact angle using Wilhelmy method. The amino acids were analysed in their powder form after brief milling using mortar and pestle. Cover slides (24 * 24 mm) were covered by double-sided tape (Scotich 12 * 1 mm) and dipped into a container of the milled amino acid to create a uniform coating. Excess powder was removed by tapping the cover slide. After measuring the perimeter (width and thickness), using a micrometer, the coated cover slide was attached to the balance loop of microbalance in the tensiometer (QCT-100 Interfacial Tensiometer, Camtel Ltd., UK). The beaker under the sample was filled with 75 ml double distilled water at temperature of 25 °C (liquid medium).

The computer was programmed to lower the sample to a distance of 10 mm after contact with the liquid medium at a constant speed of 0.20 mm/s. The contact angle was calculated automatically (using Wilhelmy equation) at regular interval and recorded as a function of time.

*2.2.7. Morphological examination*

The inner structural morphology and pore size of the freeze-dried tablets were examined by scanning electron microscopy (SEM, STEREOSCAN 90, Cambridge Instrument). Thin horizontal cross-section sample was prepared by cutting the tablet with a razor blade. The samples were placed onto double-sided adhesive strip on an aluminium stub. The specimen stub was coated with a thin layer of gold using a sputter coater (Polaron SC500, Polaron Equipment, Watford, UK) at 20 mA for three 3 min and then examined by SEM. The acceleration voltage (kV) and the magnification can be seen on each micrograph.

*2.2.8. Statistical analysis*

The effect of inclusion amino acids on the glass transition temperature of the formulation in the frozen state was compared to those of the control (composed of gelatin only) and against each other using one-way analysis of variance with Dunnett multiple comparison test and one-way analysis of variance with Tukey–Kramer multiple comparison test, respectively. The hardness, fracturability and disintegration of the lyophilised tablets after inclusion of the amino acids were statistically compared to those of the control (composed of gelatin only) using one-way analysis of variance with Dunnett multiple comparison test. The total porosity of the tablets and the wetting parameters of the amino acids were compared against each other using one-way analysis of variance with Tukey–Kramer multiple comparison test. The significant level was 0.05.

## 3. Results and discussion

### 3.1. Thermal analysis

Thermal analysis of the frozen formulations is crucial in the development of lyophilised tablets to ensure the formation of intact tablets with minimal morphological defects and also to determine the molecular state of the excipients (amorphous or crystalline). Measurement of glass transition temperature of maximally freeze concentrated ($T'_g$) solution reflects the molecular mobility of the excipients as a function of temperature within the frozen matrix which in turn dictates the stability of the formulation during the lyophilisation process. Freeze drying of formulations at temperatures 1–3 °C above their $T'_g$ (collapse temperature, $T_c$) usually induces physical collapse due to the increase in the mobility of the frozen solution [17]. Accordingly, to protect the formulation matrix from possible collapse, the temperature of the freeze-dried product should not exceed the collapse temperature, and a safety margin is required between the two temperatures (2–5 °C) to ensure the reproducibility of the process [18]. This has a direct impact on the freeze-drying regime, as lower shelf temperature is required to successfully freeze dry formulations comprising of low $T'_g$, which in turn prolongs the primary drying time significantly [19]. In addition, crystallisation during the freeze-drying stages (freezing, annealing or primary drying) is believed to give more stability to the formulation, protect against possible collapse and produce elegant lyophilised product [1,5]. Therefore, excipients that crystallise during the freeze-drying process are more suitable as bulking agents [20]. However amorphous materials (lyoprotectant) are also required in the lyophilised formulation to replace the sublimed water molecules and consequently protect against any structural changes or aggregation in the final product (lyoprotectant) [21].

The thermal properties of frozen aqueous solutions containing 5% gelatin and various concentrations of amino acids are summarised in Table 1. Limitations in the aqueous solubility of some amino acids prevented them from undergoing thermal analysis at higher concentration. At concentration of 10% w/w (total solid) of amino acids, the tested formulations showed thermal step in the baseline, glass transition of maximally freeze-concentrated sample ($T'_g$), of the heating scan, indicating that the formulations remained in amorphous state during the freezing, annealing and heating processes. Given that the $T'_g$ of the control (5% gelatin without amino acid) was $-11.72 \pm 0.72$ °C ($n = 3$), addition of 10% w/w of the different amino acids significantly lowered the $T'_g$ of the formulation (one-way ANOVA/Dunnett: $\rho < 0.05$). The lowest $T'_g$ was recorded for alanine and proline. The decrease in the glass transition of the formulations was possibly due to the plasticizing effect of the amino acids. This is in line with previously reported research, which has shown that freeze-dried systems upon inclusion of solutes within the formulation result in lowering of the glass transition temperature and is dependent on the interactions between the added excipient and unfrozen water [22]. Addition of plasticizing agents potentially reduces the intermolecular forces between binder molecules and increases polymer chain mobility, thereby providing a cushioning effect. However, the degree of plasticizing varied between the amino acids, which can be attributed to the differences in their physicochemical properties [23] and total number of moles added.

In order to further understand the differences, a plot between the molecular weight and plasticizing effect of amino acids on gelatin solution was drawn (Fig. 1). The low correlation coefficient ($R^2 = 0.695$) was probably due to the role of other physicochemical properties such as solubility and viscosity [23]. However, a general trend which showed that low molecular weight amino acids had a higher plasticizing effect was observed (Fig. 1). This could be a con-

**Table 1**
The glass transition temperature of maximally freeze concentrated ($T'_g$) and crystallisation event of 5% gelatin solution in water with 10, 30, 50 and 70 % (of total solid material) of amino acids (mean ± SD, $n = 3$)

| Amino acid | 10% | | 30% | | 50% | | 70% | |
|---|---|---|---|---|---|---|---|---|
| | $T'_g$ (°C) | Cryst. (°C) | $T'_g$ (°C) | Cryst. (°C) | $T'_g$ (°C) | Cryst. (°C) | $T'_g$ (°C) | Cryst. (°C) |
| Alanine | −21.55 ± 0.50 | * | −36.68 ± 0.15 | * | −12.85 ± 0.22 | −32.77 ± 0.43 | * | −40.11 ± 0.80 |
| Arginine | −14.46 ± 0.27 | * | −21.36 ± 0.13 | * | −27.32 ± 0.21 | * | −32.60 ± 0.09 | * |
| Threonine | −18.51 ± 0.11 | * | −30.21 ± 0.45 | * | −35.41 ± 0.37 | * | −38.61 ± 0.49 | * |
| Glycine | −20.46 ± 0.17 | * | −12.51 ± 0.82 | −28.81 ± 0.85 | * | −45.53 ± 0.52 | * | −32.32 ± 1.00 |
| Cysteine | −17.22 ± 0.69 | * | −25.01 ± 0.39 | −10.33 ± 0.40 | −13.14 ± 0.29 | −23.01 ± 0.40 | * | * |
| Serine | −18.75 ± 0.22 | * | −25.70 ± 0.58 | * | −12.56 ± 0.18 | −16.98 ± 0.33 | * | −24.00 ± 0.53 |
| Histidine | −16.25 ± 0.41 | * | −21.34 ± 0.13 | * | −24.59 ± 0.30 | * | – | – |
| Lysine | −20.34 ± 0.20 | * | −34.63 ± 0.63 | * | −39.08 ± 0.21 | * | −46.84 ± 0.22 | * |
| Valine | −19.09 ± 0.17 | * | −12.02 ± 0.26 | −24.25 ± 0.44 | * | * | – | – |
| Asparagine | −16.82 ± 0.28 | * | −21.90 ± 0.16 | * | – | – | – | – |
| Glutamine | −17.57 ± 0.60 | * | −24.84 ± 0.14 | * | – | – | – | – |
| Proline | −21.47 ± 0.51 | * | −37.05 ± 0.86 | * | −50.43 ± 0.30 | * | >−65 | * |

(*) No event detected, (–) not soluble.

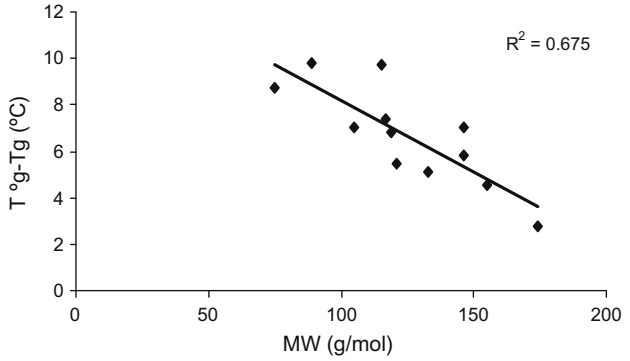


**Fig. 1.** The effect of molecular weight of amino acids on the glass transition temperature of frozen solutions containing 5% aqueous gelatin solution at concentration of 10% w/w (total solids) of the tested amino acids. $T'_g$: glass transition temperature of maximally freeze-concentrated sample of 5% gelatin with 10% amino acid, $T'_g$: glass transition of maximally freeze-concentrated sample of 5% gelatin solution.

sequence of the higher number of amino acid moles provided by the low molecular weight amino acid in the formulation, as all the amino acids were added to the formulation mixture as a weight per weight percent. The presence of larger number of particles within the formulation may eventually have a higher cushioning effect resulting in greater decrease of intermolecular forces between the gelatin and gelatin-water molecules.

Upon increase of concentration to 30% w/w, all the tested amino acids showed significant reduction in their $T'_g$ values when compared to their 10% formulation (one-way ANOVA/Dunnett: $\rho < 0.05$) except glycine and valine where partial crystallisation was observed (Table 1). At this concentration, the amino acids that showed lower $T'_g$ values appeared to retain their amorphous state throughout the heating range (−65 to 20 °C) except glycine, cysteine and valine, where partial crystallisation was observed. However, at a concentration of 50% w/w alanine, serine, glycine, cysteine and valine exhibited crystallisation, whereas the rest of the amino acids retained their amorphous state in the formulation during the cooling, annealing and heating processes as demonstrated by $T'_g$ values (Table 1). At the highest studied concentration (70% w/w), arginine, threonine, lysine and proline retained their amorphous state. The ability of arginine to preserve the amorphous behavior in the freeze concentrated solutions has previously been documented by Izutsu et al. [24], studying the effect of counterions on the physical properties of arginine in frozen solutions and freeze-dried solids. Although there was no event detected in 70% proline formulations, it can be anticipated that the glass transition was below the heating range employed (−65 to 20 °C), based on the data recorded for lower concentrations where lowering of the glass transition was noted upon increase of proline concentration. On the other hand, the crystallisation behavior of alanine, glycine, serine and cysteine prohibited the formulations from undergoing any glass transition event at this high concentration (Table 1).

Freeze drying of the formulations in this study using the applied regime (primary drying for 48 h at shelf temperature of −40 °C and secondary drying for 10 h at shelf temperature of 20 °C and vacuum of 50 mTorr) revealed that the formation of intact tablets (with no signs of morphological defect) was crucially influenced by the above thermal properties of the formulation. All the formulations that showed tendency to crystallise formed elegant tablets with no signs of morphological defect regardless of their $T'_g$ temperatures, which confirms the role of readily crystalline excipient in the formulation of lyophilised RDTs as discussed above. For amorphous formulations, the formation of intact tablet was dependant on $T'_g$. Formulations with $T'_g$ lower than −40 °C showed major structural collapse after freeze drying, while intact tablets were formed from higher $T'_g$. In the case of 30% proline formulation, partial collapse was noticed possibly due to the narrow safety margin between the shelf temperature and $T'_g$, therefore these tablets were excluded from further characterisation.

### 3.2. Porosity

The porosity of the RDTs at amino acid concentrations of 10%, 30%, 50% and 70% (w/w) is summarised in Table 2. The results suggested that each increment in the concentration of the amino acid in the RDTs was associated with a significant decrease in the total porosity ($\rho < 0.05$), possibly due to a decrease in the water concentration in the stock solution (because water is the porogen element in the formulation). The results, also, showed that inclusion of different amino acids at concentration of 10% (w/w) produced tablets with insignificant differences in their total porosity ($\rho > 0.05$). However, at higher concentrations (30%, 50% and 70% w/w) of amino acids some variations in the total porosity were noticed. As all tablets in this study were produced using the same procedure and the same binder stock solution, any differences in their porosity were attributed to the inclusion of amino acids and their concentration. Tablets based on the same concentration of alanine, arginine, threonine, serine, cysteine, histidine and asparagine had very close total porosity values (less than 2% variation), whereas tablets fabricated from glycine and lysine at similar concentration produced tablets with slightly lower total porosity ($\rho < 0.05$), and even much lower porosity was displayed by valine and glutamine formulations ($\rho < 0.001$) when compared to the rest of the amino acids. Further discussion about the impact of porosity on RDT characteristics is described in the sections of mechanical properties and mechanism of disintegration (below).

### 3.3. Mechanical properties

One of the inherent issues associated with the formulation of lyophilised rapid disintegrating tablets is the weak mechanical properties [13,25,26] with the consequence that additional protection in the form of specialized packaging is required for the tablet to withstand mechanical stresses during shipping, storage and handling by patients. The poor mechanical properties are as a result of the porous anatomical architecture of the lyophilised RDT consisting of a three-dimensional network of binder molecules (see Fig. 2). Previous research has shown that the two common methods to enhance the mechanical strength of the lyophilised RDTs are the inclusion of higher concentration of the binder and addition of excipients such as matrix supporting agents (saccharides and polyols) [5]. However, increase in binder concentration

**Table 2**
The total porosity of RDTs based on 10%, 30%, 50% and 70% (w/w) amino acids.

| Amino acid | Porosity (%) | | | |
|---|---|---|---|---|
| | 10% | 30% | 50% | 70% |
| Alanine | 96.01 ± 0.32 | 94.12 ± 0.13 | 91.37 ± 0.15 | 86.12 ± 0.41 |
| Arginine | 95.84 ± 0.41 | 94.09 ± 0.27 | 90.70 ± 0.22 | 85.31 ± 0.52 |
| Threonine | 95.92 ± 0.22 | 94.36 ± 0.24 | 92.76 ± 0.23 | 86.61 ± 0.54 |
| Glycine | 95.43 ± 0.35 | 92.42 ± 0.23 | 88.14 ± 0.21 | 82.03 ± 0.40 |
| Cysteine | 96.12 ± 0.45 | 94.67 ± 0.27 | 92.71 ± 0.31 | 86.31 ± 0.35 |
| Serine | 96.47 ± 0.30 | 95.00 ± 0.24 | 93.14 ± 0.32 | 87.83 ± 0.29 |
| Histidine | 95.79 ± 0.43 | 94.14 ± 0.20 | 92.64 ± 0.30 | – |
| Lysine | 95.21 ± 0.27 | 92.45 ± 0.31 | 88.21 ± 0.15 | * |
| Valine | 95.12 ± 0.25 | 88.49 ± 0.27 | 74.94 ± 0.34 | – |
| Asparagine | 96.35 ± 0.12 | 94.94 ± 0.35 | – | – |
| Glutamine | 95.17 ± 0.50 | 87.21 ± 0.62 | – | – |
| Proline | 96.10 ± 0.18 | * | * | * |

(*) No event detected, (–) not soluble.

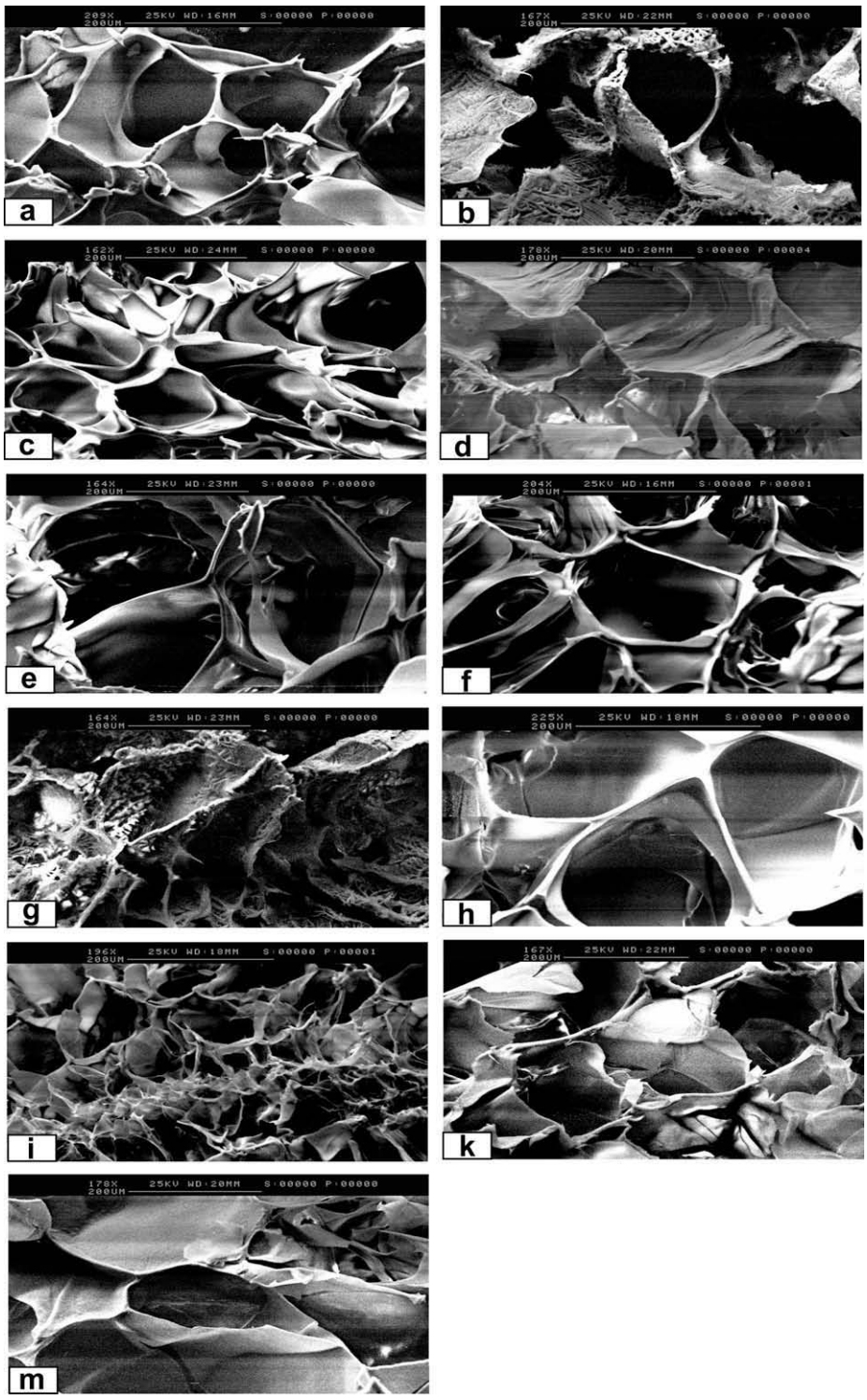


**Fig. 2.** Scanning electron micrograph of RDTs based on: (a) 50% arginine, (b) 50% valine, (c) 50% lysine, (d) 50% alanine, (e) 50% threonine, (f) 50% serine, (g) 30% glutamine, (h) 50% histidine, (i) 50 % cysteine, (k) 30% asparagine and (m) 50% glycine.

has a detrimental effect on the disintegration time of the tablets due to increase in intermolecular attraction between the binder molecules resulting in retardation in disintegration time profile leaving the incorporation of matrix supporting agents as a more pragmatic method.

In this study, the use of 5% (w/w) gelatin stock solution as a binder proved to give the RDTs high resistant to friability, less the 0.15% (data not shown). However, due to the highly porous structure, the RDTs have a spongy nature, which is easy to deform in response to external forces. Therefore, the effect of inclusion of varied concentration of amino acids on the mechanical properties of the tablets was evaluated by applying a compression force through a 5-mm-diameter probe, and the peak force after 1-mm compression was taken as the hardness.

The hardness of the RDTs after inclusion of varied concentration of amino acids is presented in Fig. 3. The results showed that inclusion of amino acids at low concentration of 10% and 30% w/w (total solid) did not improve the hardness of the tablets significantly

when compared to gelatin only formulation (one-way ANOVA/Tukey–Kramer: $\rho > 0.05$). However, upon increase of concentration to 50%, alanine ($\rho < 0.01$), arginine ($\rho < 0.05$), threonine ($\rho < 0.05$), glycine ($\rho < 0.05$) and serine ($\rho < 0.01$) significantly (one-way ANOVA/Tukey–Kramer) improved the hardness of the tablets from 13.5 ± 0.7 N for gelatin only tablet (control) to 18.3 ± 1.0 N, 17.5 ± 1.8 N, 20.3 ± 1.2 N, 18.1 ± 0.9 N, 19.7 ± 1.5 N and 22.2 ± 1.7 N, respectively. At the highest studied concentration (70% w/w), only tablets based on arginine, glycine and serine achieved progressive enhancement in hardness over their 50% formulation, with the highest hardness recoded by the serine formulation (37.0 ± 4.5 N).

Generally, the mechanical properties of tablets are mainly influenced by the intermolecular bonding force and contact points between the excipients [27]. The extent of contact between the matrix forming agents within the lyophilised RDTs is influenced by the total porosity of the tablets, decreasing the porosity increases the contact points between the matrix forming agents within the RDT. Accordingly, the improvement in the mechanical properties of the RDTS upon increasing the concentration of amino acids in the formulation was a result of decreasing the porosity (see porosity results). However, the degree of improvement was varied between the amino acids as a consequence of their variation in the molecular interaction with the binder (gelatin). For instance, although valine and glutamine formulation had the lowest porosity values (higher contact points), no improvement in the hardness was achieved, and even significant deteriorations were noticed in the 10% glutamine and 50% valine formulations when compared to the control, which suggests weak bonding interaction of these amino acids with gelatin fibres. These data appear to be supported by scanning electron microscopy (SEM) images (Fig. 2) of the inner structure of RDTs, which show that valine (Fig. 2b) and glutamine (Fig. 2g) molecules deposited at the surface of gelatin fibres instead of integrating within the fibre suggesting incompatibility of these amino acids with gelatin. On the other hand, SEM images of tablets based on amino acids that improved the hardness show homogenous network of fibres without any segregation/deposition of particles on the surface, suggesting that these amino acids integrated completely with gelatin fibre and consequently added extra support to the tablet structure.

### 3.4. Disintegration time

The results from the disintegration study are summarised in Fig. 4. As expected, the disintegration profile of the RDTs was distinctive for each amino acid (Fig. 4), possibly due to differences in

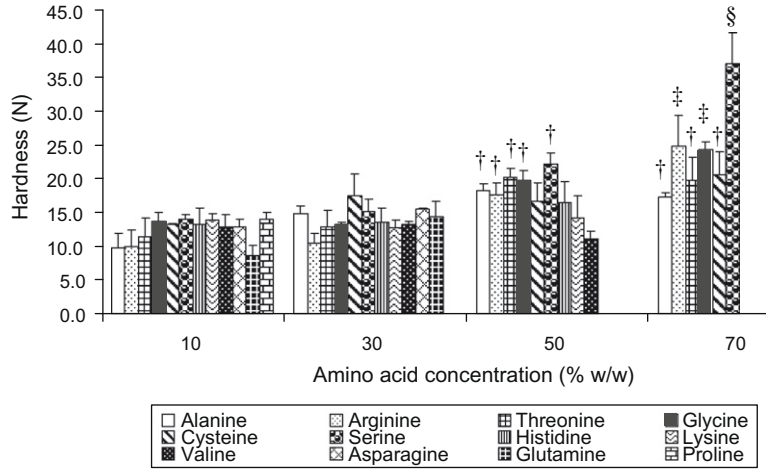


**Fig. 3.** The effect of varied concentration of amino acids on the hardness of lyophilised tablets based on 5% gelatin solution. Results are mean ± SD, $n = 3$. Statistical difference (one-way ANOVA/Tukey–Kramer) from control: $^{\dagger}\rho < 0.05$, $^{\ddagger}\rho < 0.01$, $^{\S}\rho < 0.001$.

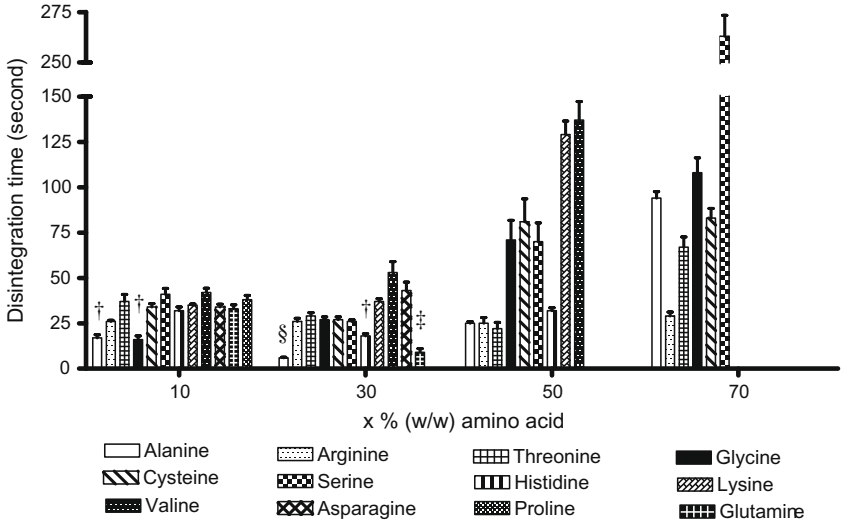


**Fig. 4.** The disintegration time of tablets based on 5% gelatin stock solution after inclusion of varied concentration of amino acids. Results are mean ± SD, $n = 3$. Statistical difference (one-way ANOVA/Tukey–Kramer) from control (shorter): $^{\dagger}\rho < 0.05$, $^{\ddagger}\rho < 0.01$, $^{\S}\rho < 0.001$.

**Table 3**
The wetting properties of the amino acids that showed complete wetting. Results are mean ± SD, $n = 5$.

| Amino acid | Phase transition time (s) | Wetting time (s) |
|---|---|---|
| Proline | 0 | 1.3 ± 0.6 |
| Alanine | 1.0 ± 0.7 | 15.8 ± 3.7 |
| Glycine | 0 | 20.0 ± 3.2 |
| Arginine | 4.9 ± 2.1 | 27.5 ± 1.2 |
| Threonine | 0 | 34.3 ± 1.5 |
| Cysteine | 0 | 25.5 ± 2.2 |
| Asparagine | 0 | 42.9 ± 0.3 |

their physicochemical characteristics. At concentration of 10% (w/w), all of the tested amino acids showed no improvements on the disintegration time when compared to 5% gelatin formulation except alanine and glycine, which decreased the disintegration significantly (one-way ANOVA/Tukey–Kramer: $\rho < 0.05$), from 29 ± 2 s for the 5% gelatin formulation to 17 ± 3 s and 16 ± 4 s, respectively. By increasing the concentration to 30% (w/w), alanine progressively promoted the disintegration profile to 6 ± 1 s, which was the shortest disintegration time in the current study, whereas glycine showed a significant deterioration when compared to its 10% formulation ($\rho < 0.05$). Interestingly, tablets based on 30% histidine and glutamine achieved significantly shorter disintegration times compared with their 10% counterparts and control (5% gelatin) and recorded disintegration times of 18 ± 2 s ($\rho < 0.05$) and 9 ± 4 s ($\rho < 0.01$), respectively. The rest of the tested amino acids continued their trends by not offering any improvement over the disintegration time of the control (Fig. 4). Inclusion of higher concentration of amino acids (50% and 70% (w/w)), seemed to have negative effect on the disintegration profile of the tablets, except in case of arginine, where the disintegration profile seemed to be independent of concentration (one-way ANOVA/Tukey–Kramer: $\rho > 0.05$).

### 3.5. Wettability and wetting time

The wettability of compressed RDT formulations has been investigated and correlated with the disintegration profile in previous research [27,28]. However, in the case of lyophilised RDT, measurement of the wetting process of the whole tablet is extremely difficult due to the very short disintegration time of the tablets.

In the current study, all the RDTs were formulated by adding amino acids individually at varied concentration to a fixed concentration of gelatin stock solution (5% w/w). Therefore, the disintegration time of the RDTs is believed to be influenced by both the concentration and wetting properties of the amino acid. Accordingly, the wetting profiles of the tested amino acids in the powder form were investigated and correlated with the disintegration time of the RDTs.

Measuring the wettability (expressed as contact angle) of pharmaceutical powder requires precision in sample preparation and is associated with extreme experimental care [29]. Among the different techniques available, the Wilhelmy method which uses powder coated glass slides as a measurement plate has been shown to demonstrate superior reproducibility and accurate measurement of contact angle [30] (Table 3).

The contact angle ($\theta$) profiles of the tested amino acids are presented in Figs. 5 and 6. Valine displayed the highest contact angle which increased steadily with time until it was stabilized on an average of 147 ± 5 ($n = 5$), indicating that valine is not wettable in water ($\theta > 90°$). Serine, lysine, glutamine and histidine showed partial wetting profile ($90° < \theta < 0°$) with average contact angle values of 50 ± 3°, 39 ± 2°, 27 ± 4° and 23 ± 3° ($n = 5$), respectively (Fig. 5). The rest of the tested amino acids (alanine, arginine, threonine, glycine, cysteine, asparagine and proline) displayed complete wetting profile (zero contact angle) (Fig. 6). To differentiate between the wettability profiles of these amino acids, two parameter were identified: the phase transition time, which is the time required for phase transition from partial ($90° < \theta < 0°$) to complete wetting ($\theta = 0°$), and wetting time, the time taken for the complete wetting phase to finish, which appears in the wettability profile as sudden increase in the contact angle (Fig. 6). This increase in the contact angle is caused by the exposure of the adhesive layer to the water (wetting medium) as the tested powder starts to depart the plate into the liquid medium [30]. The summary of the two parameters is presented in Table 4. The results revealed that proline, threonine, glycine, cysteine and asparagine showed complete wetting without delay (phase transition time = 0 s), whilst alanine and arginine required 1.0 ± 0.7 s and 4.9 ± 2.1 s, respectively, to display complete wetting. On other hand, proline displayed the shortest wetting time of 1.3 ± 0.6 s, followed by alanine, glycine, cysteine, arginine, threonine and asparagine (Table 4). Interestingly, alanine, which is classified as hydrophobic amino acid, had shorter wetting time than arginine, threonine and asparagine, which are known to be more hydrophilic. The shorter wetting time of alanine compared to higher hydrophilic amino acids has been previously reported [31].

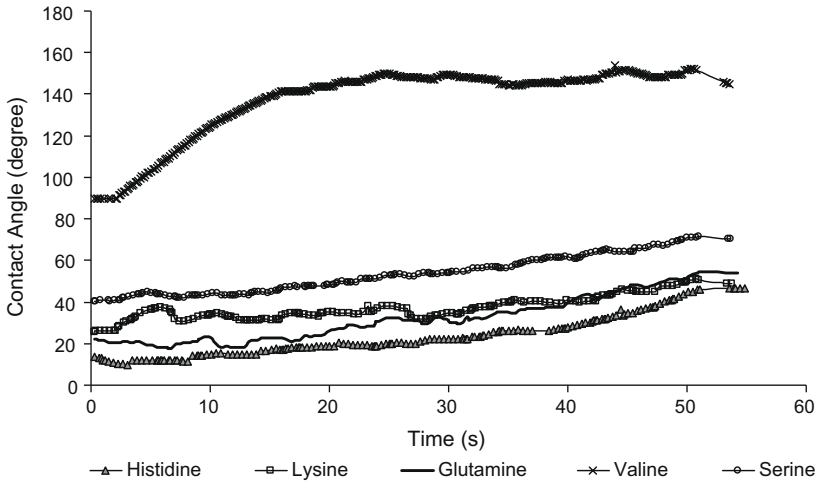


**Fig. 5.** Representative profiles of contact angles of water on poorly and partially wettable amino acids as a function of time.

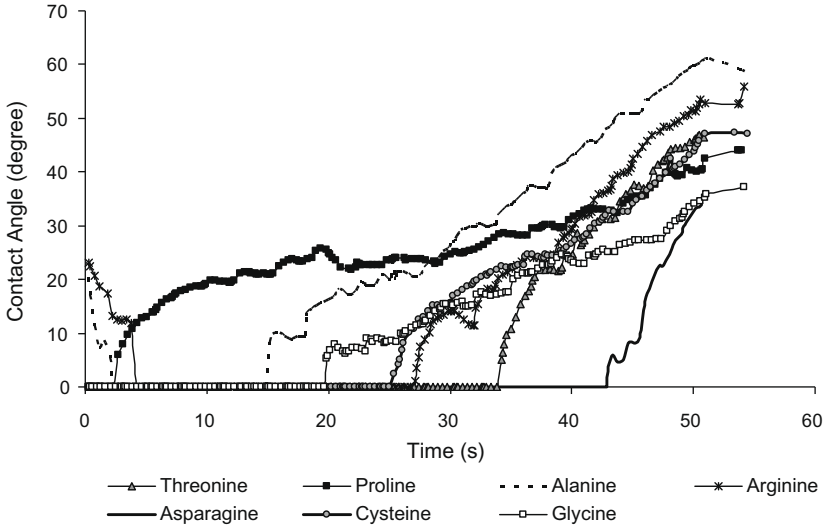


**Fig. 6.** Representative profiles of contact angles of water on highly wettable amino acids as a function of time.

### 3.6. Mechanism of disintegration

In this study, the disintegration time profiles of the RDTs as a function of amino acid concentration (Fig. 4) were analysed depending on the wetting profile of the incorporated amino acids in order to determine the factors that influence the disintegration of the RDTs and consequently understand the mechanism of disintegration. In the case of poorly wettable amino acid (valine), the inclusion of higher concentration of valine in the formulation deteriorated the disintegration time possibly due to the decrease in the total porosity and creation of matrix that interacts less favorably with water (low wettability). For highly wettable amino acids (alanine, arginine, threonine, glycine, cysteine and asparagine), parabolic relationships between the disintegration time and the concentration of amino acid were seen but with different dip values (shortest disintegration time) that were obtained at distinct concentrations for each amino acids (Fig. 4). This parabolic relationship may possibly be due to the inclusion of highly wettable amino acid within the formulation of RDTs which enhances the interaction of tablet's matrix with water (disintegrating medium) but, at the same time, decreases the porosity which inhibits water penetration into the tablet. Therefore, each amino acid exhibited a decrease in disintegration time at an optimal concentration where a balance between porosity and high wettability was created and consequently achieved the shortest disintegration time. Fig. 7 represents a correlation between the wetting time of these highly wettable amino acids and average disintegration time of RDTs. The linearity of the correlation observed suggested that the wetting time of the amino acid plays an important role in determining the disintegration time of RDTs. However, this role is seemed to be highly affected by the porosity of the RDTs. For instance, the correlation between the wetting time and disintegration time for RDTs based on 50% amino acids was poor, due to different porosity of the RDTs at this concentration (Table 4). Accordingly, tablet's porosity and wetting time of the amino acid play a major role in determining the disintegration time. This mechanism of disintegration usually referred as wicking is due to weakening of the intermolecular bonds upon penetration of the disintegration medium between the tablet's excipients and consequently resulting in complete disintegration of the tablets.

On the other hand, partially wettable amino acids (serine, lysine, glutamine and histidine) showed a mix of the two previous profiles. The amino acid with lower contact angle (higher wettability), such as glutamine and histidine, mimicked the highly wettable amino acid profiles, and a parabolic relationship with the concentration was noticed, whereas amino acids with higher contact angle, such as serine and lysine, followed the trend of poorly wettable amino acids as increasing their concentration in the RDT profoundly increased the disintegration time.

**Table 4**
The lyophilised tablet index of tablets based on 5% gelatin stock solution and varied concentration of amino acids.

| Amino Acid | LTI | | | |
|---|---|---|---|---|
| | 10% | 30% | 50% | 70% |
| Alanine | 1.26 | 4.99 | 1.58 | 0.39 |
| Arginine | 0.82 | 0.85 | 1.47 | 1.84 |
| Threonine | 0.66 | 0.95 | 1.96 | 0.63 |
| Glycine | 2.54 | 1.01 | 0.59 | 0.48 |
| Cysteine | 0.82 | 1.35 | 0.44 | 0.53 |
| Serine | 0.72 | 1.26 | 0.67 | 0.30 |
| Histidine | 0.87 | 1.63 | 1.10 | 1.73 |
| Lysine | 0.84 | 0.73 | 0.23 | * |
| Valine | 0.65 | 0.54 | * | * |
| Asparagine | 0.79 | 0.77 | * | * |
| Glutamine | 0.55 | 3.39 | * | * |
| Proline | 0.78 | * | * | * |

### 3.7. The lyophilised tablet index

In order to evaluate the effect of inclusion of amino acids on the hardness and disintegration at the same time and compare it to the gelatin only formulation (control), lyophilised tablets index (LTI) values were calculated according to the following equation:

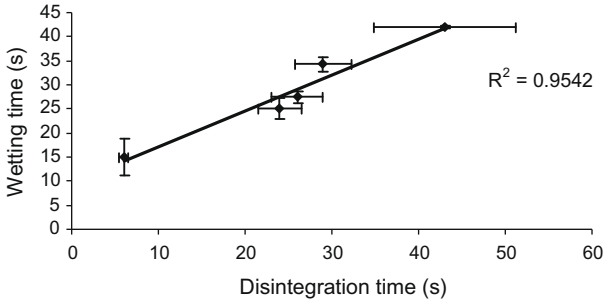


**Fig. 7.** Relationship between wetting time of the amino acids and disintegration time of the RDTs.

$$LTI = (H/DT) \div (H^{\circ}/DT^{\circ})$$

where H: hardness of the tested tablet, DT: disintegration time of the tested tablet, H°: hardness of the control tablets, DT°: disintegration time of the control tablet.

The LTI value provided a ratio indicative of whether the prepared amino acid formulation was better than the gelatin only formulation [5]. Values greater than 1 indicate improvements over the gelatin formulation, whereas lower values suggest retardation in the overall tablet properties (disintegration time and hardness). In addition, LTI values can be used to rank the improvements in tablet properties among various formulations. The results (Table 4) revealed that alanine, glutamine, glycine, arginine, histidine, serine and threonine were able to improve the overall tablets properties to different extent at different concentration. Alanine achieved the highest value at concentration of 30% (w/w) with LTI value of 4.99, followed by the 30% glutamine formulation (LTI = 3.39) and then the 10% glycine (LTI = 3.39). Previous research from our laboratory investigating the influence of inclusion of saccharides and polyols as matrix supporting/disintegration enhancing agents on the overall tablet properties using 5% gelatin of low bloom strength as a binder (similar conditions to the current study) [32] has shown that LTI values ranged between 0.52 and 8.10, which are comparable to the LTI values from this current study, demonstrating the suitability of the amino acids in the formulation of RDTs.

## 4. Conclusion

The current study suggests that successful formulation of saccharides-free lyophilised RDTs requires amino acids that crystallise in the frozen state or display relatively high $T'_g$, interact and integrate completely with the binder and, also, display short wetting time with the disintegrating medium. The tested amino acids have showed varied capability to fulfil all the required characteristics for the formulation of lyophilised RDTs. However, inclusion of an optimised concentration of alanine achieved the best balance and therefore produced RDTs with superior characteristics.

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