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The influence of chitosan and sodium alginate and formulation variables on the formation and drug release from pellets prepared by extrusion/spheronisation

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Abstract

The influence of the incorporation of two oppositely charged hydrophilic natural polymers, chitosan and sodium alginate, alone and in combination, on the ability of formulations containing a model drug (paracetamol) to form spherical pellets by the process of extrusion/spheronisation and the properties of the pellets, has been undertaken. A statistically experimental design was employed to allow the major factors which determined the properties of the pellets, to be identified. A standardised procedure was used to prepare the pellets with a ram producing the extrudate for spheronisation. Statistical analysis of the results indicated that the formulation variables of the type and level of the polymer, the proportion of the model drug, and the proportion of the microcrystalline cellulose influenced (a) the quantity of liquid binder required to produce a good formulation (narrow size range and high value for the shape factor indicating sphericity), (b) the steady-state extrusion force, (c) the pellet perimeter, (d) the apparent pellet density and (e) the porosity of the pellets. The median size of the pellets of the "good formulation" could only be related to the chitosan and sodium alginate content of the formulations. The proportion of the drug, chitosan and sodium alginate content of the formulation significantly influenced the in vitro dissolution of the model drug (paracetamol). The drug release mechanism differed with the formulation variables, although if the pellets remained intact during the dissolution test, diffusion was the controlling mechanism. There was no significant advantage to be gained by using a mixture of the two polymers in terms of retarding drug release.

Keywords: Drug release; Extrusion/spheronisation; Hydrophilic polymers (chitosan, sodium alginate); Pellet properties

1. Introduction

The preparation of pellets by extrusion/spheronisation is a well-documented process. As yet however, there are only general guidelines as to the range of factors, which are involved in ensuring that a successful product can be achieved. There are also no absolute criteria as to what is and what is not a successful product. In particular what is accepted as a satisfactory 'sphericity' is not agreed, nor even a standard method of measuring 'sphericity'. There is also a range of variations in the process, which adds to the confusion in the literature when it comes to judging whether or not an approach to solving a formulation is appropriate. The main variations in the process are the type of mixing process used to incorporate the liquid into the powder and the extrusion stage, where

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the options available can have an appreciable effect on the outcome of the process. The main types of extruders available are described by Newton (2002) and range from the screen extruders, which have 'punched' holes whose length approximates to their diameter to 'drilled' holes, whose length is usually at least double that of the diameter. The former provides limited densification of the extrudate while the latter ensures significant densification. The extrudates produced by these two types are not the same and respond differently to the spheronisation stage, the former being much more influenced by the rotational speed and load on the plate than the latter. Thus in considering the literature associated with the influence of formulation variables, it is important to know the details of the processes involved and methods of assessment of the properties of the pellets produced, before the influence of the formulation variables can be assessed. Unfortunately, these are not always available or when they are, fail to appreciate the importance of a key issue. Thus, for example, a recent paper by Heng and Koo (2001) writes about the influence of formulation variables on the process of extrusion/spheronisation when the wet powder mass is not extruded but merely pushed by hand through a sieve, rather like the process of wet granulation. The influence of the spheronisation stage on such a wet mass will be entirely different to that associated with the processing of an extrudate formed by forcing through a long die. There are certain formulations, which will form pellets irrespective of the extrusion stage, as was demonstrated by Chopra et al. (2001), who described a formulation, which produced spherical pellets when the wet mass from the planetary mixer was placed directly on the spheroniser plate. In other cases, however, formulations can be very sensitive to the processing stages and the ingredients. Of particular concern is the quantity of water added, it's method of incorporation into the powder and the details of the extrusion process.

A major commercial use of pellet formulations produced by extrusion/spheronisation, is in the field of controlled release formulations, where their spherical from and the potential to incorporate relatively high drug levels, lends them to the preparation of film-coated products. If however, as is the case with tablets, pellets could be formulated to provide a control of release without the need to film coat, then cost savings in production could be achieved. In tablets such effects are achieved by adding hydrophilic polymers, applying direct compression to avoid the swelling that would occur if an aqueous granulation was used in the preparation of the tablets. Incorporation of swelling polymers into the process of extrusion/spheronisation could present problems due to the inclusion of water in most formulations. Even if the polymers were not grossly swelling, the increase in viscosity, which occurs with hydrophilic polymers could significantly change the consistency of the wet powder mass to prevent processability. Just how the presence of polymers within the microcrystalline cellulose provides appropriate rheological properties to ensure good smooth extrudate is produced, has been quantified by Raines et al. (1990). Unfortunately, the ability to quantify the rheological properties required to ensure a satisfactory process of pellet production has yet to be achieved. Boutell et al. (2002) reported that doubling the viscosity of the binder liquid by the addition of the Newtonian fluid glycerol did not hinder pellet formation. O'Connor and Schwartz (1985) reported that when sodium carboxymethyl cellulose was incorporated into the microcrystalline cellulose (Avicel RC581 and CL611) and used in the formulations pellet production and retardation of drug release was possible. Ghali et al. (1989) also found that if the RC581 grade of MCC was added to the normal PH101, then satisfactory pellets could be produced with retardation of drug release. Both these studies used screen extruders. Newton et al. (1992), however, found that these grades of MCC did not readily form good spherical pellets in spite of extruding well through long dies.

In a study involving a range of binders on formulation of pellets containing 80% of theophylline, 18% MCC and 2% solid binder, Funck et al. (1991) found that pellets were less friable when binder was present than absent, but there was no marked difference in the drug release properties. Again, a screen extruder produced extrudates, as they were in the study by Goskonda and Upadrashta (1993), who studied the influence of the incorporation of chitosan of different grades and quantities, combined with Avicel RC581 to provide drug loadings of 20% paracetamol or theophylline. The pellets were characterised in terms of their bulk and tapped densities, friability and size (by sieving). There was however no assessment of shape, although SEM pictures illustrated that the pellets were 'round' but had very irregular surfaces. The in vitro

drug release profiles were found to be formulation and dissolution media dependent. Tapia et al. (1993) incorporated chitosan dissolved in glacial acetic acid in pellet formulations. Pellets could be produced with up to 3% chitosan, extruding through long dies (L/R > 4). Above this level, extrusion/spheronisation was not possible. The slowest drug release was found if pellets of 2 mm diameter were prepared.

Law and Deasy (1997) found that incorporating PVP and sodium lauryl sulphate improved the preparation of pellets containing indomethacin when a long die extruder was used to produce the extrudate. They claimed that the in vitro drug release profiles were better than a commercial brand of a controlled release formulation. Goskonda and Upadrashta (1993) claimed that it was possible to produce pellets that retarded drug release by the incorporation of polymer dispersions normally used in film coating. Here screen extrusion was used to produce the extrudate. While the pellet size was recorded there was no assessment of pellet shape. Neau et al. (1996) reported that, by careful selection of the added electrolyte and its concentration, pellets containing 5% chlorpheniramine maleate could be produced containing different levels of Carbopol 975P. They used a screen extruder to produce the extrudate. The authors admitted that the roundness of the pellets was not satisfactory even when measured with an insensitive method of assessing roundness. The Carbopol content, the pH and the ionic strength of the dissolution media influenced the in vitro drug release.

Thus, there are differences in the outcome of the incorporation of hydrophilic polymers into the formulation of pellets produced by extrusion/spheronisation. Therefore to add to the understanding of the formulation possibilities, a system containing a polyanionic polymer (sodium alginate) and a polycationic polymer (chitosan) and mixtures thereof, have been assessed in terms of their influence on the ability to produce pellets, and the properties of the pellets produced.

2. Materials and methods

2.1. Materials

Paracetamol of EP quality was obtained from Rhone-Poulenc, Poissillon, France (Batch no. 941 3425) and had a number mean Feret's diameter of 20.48 ± 12.43 μm, determined by an image analyser (Seescan Solitaire 512, Seescan, Cambridge, UK), connected to a black and white camera (CCD-4 miniature video camera module, Rengo Co. Toyohashi, Japan) and a microscope (Olympus BH-2, Tokyo, Japan) and an apparent particle density of 1037 kg/m^3, determined with an air comparison pycnometer (Model 930, Beckman, Irwin, USA). The chitosan had a degree of deacetylation of 78% (Seacure 252, batch number M1382 and was supplied by Proton Biopolymer A/S, Drama, Norway), had a number mean Feret's diameter of 35.54 ± 31.16 μm and an apparent particle density of 1407 kg/m^3. The sodium alginate was of EP quality, was supplied by BDH, Merck Ltd., Poole, UK, had a number mean Feret's diameter of 23.14 ± 21.04 μm and an apparent particle density of 1702 kg/m^3. The microcrystalline cellulose (MCC was Avicel PH101 batch 6521 supplied by FMC Corporation, Cork, Ireland) had a number mean Feret's diameter of 25.12 ± 18.22 μm and an apparent particle density of 1558 kg/m^3. The α-lactose monohydrate EP (batch 0100041), was supplied by Borculo Whey Products Ltd., Chester., UK, as a 'fine' grade and had a number mean Feret's diameter of 19.89 ± 11.53 μm and an apparent particle density of 1549 kg/m^3. The values for the apparent particle density of the powders were the mean of three determinations.

McIlvaine buffers, which readily provide the different pH values required were prepared freshly from stock solutions of 0.1 M citric acid and 0.2 M disodium phosphate. The dissolution media was simulated gastric juice, without the pepsin, consisting of 0.07 M hydrochloric acid and 0.034 M sodium chloride having a total ionic strength of 0.104 M. All other chemicals were 'Analar' grade supplied by Merck, Poole, UK, while the water was freshly prepared distilled water.

2.2. Methods

2.2.1. Preparation of pellets

The dry ingredients of the formulations were blended for 5 min in a planetary mixer (Kenwood Chef, Havent, Hants, UK). The appropriate quantity of binder liquid was added slowly with constant mixing, and the process continued for a further 10 min. (The process was stopped at least twice to scrape

down the sides of the bowl and the mixer blade.) The level of binder required for a formulation was determined by trial and error, as judged by the quality of the pellets, in terms of size near to the die diameter, narrow size distribution and spherical form. The quantity of binder is reported as the quantity of liquid as a percent of the dry weight of the solid in the pellet. The wet mass was extruded through a die of 1 mm diameter and 4 mm in length fitted to a barrel of a ram extruder, whose piston was driven at a cross-head speed of 200 mm/min by a Universal testing Instrument (Lloyd MX50, Lloyd Instruments, Southampton, UK). The force displacement profile was recorded to allow identification of the type of flow (Harrison et al., 1985) and the forces involved. Multiple extrusion was used to produce 250–350 g of extrudate to be spheronised for 30 min on a 22.5 mm diameter radial cut plate rotating at 1000 rpm (Caleva, Sturminster Newton, UK). The pellets were dried for 60 min at 60 °C in a fluid bed drier (Model FBD70P, R.L. Engineering Ltd., Mostyn, UK).

2.2.2. Characterisation of the pellets

For all tests other than size analysis, a random sample of pellets was selected with a spinning riffler (Microsal, Ealing, UK) from the modal fraction, unless otherwise stated.

2.2.2.1. Pellet size analysis. This was determined with a set of British Standard sieves in a root two progression of sizes between 500 and 2800 μm with 100 g of pellets by agitating for 10 min on a sieve shaker (Endecotts, Ltd., London, UK). The median diameter and the interquartile range were determined from a cumulative percentage undersize graph.

2.2.2.2. Pellet apparent density. The apparent density of the pellets was determined by an air comparison pycnometer Model 930, Beckman, Irwin, USA). The values are the mean of three determinations.

2.2.2.3. Pellet shape and outline. The two-dimensional shape factor e_R described by Podczeck and Newton (1994), the aspect ratio and the perimeter were determined with an image analyser (Seescan Solitaire 512, Seescan Cambridge, UK), connected to a black and white camera (CCD-4 miniature video camera module, Rengo Co. Ltd., Toyohashi, Japan) and a Zoom Lens (18-108/2.5 Olympus, Hamburg, Germany). The magnification was set so that one pixel was less than 26 μm and for each formulation 100 pellets were observed.

2.3. Experimental design

Five formulation variables were investigated. These were:

1. the level of paracetamol (LP), ranging from 2.5 to 40% (formulations 1–4 and 11);
2. the level of microcrystalline cellulose (LA), ranging from 30 to 70% (formulations 5–8, and 11);
3. the level of chitosan (LC) ranging from 0 to 16% (formulations 22, 26 and 27);
4. the level of sodium alginate (LSA) 0–16% (formulations 23, 28 and 29) and combinations of chitosan and sodium alginate between 0 and 4% total (formulations 1–8, 10–12, 14–17, 24 and 25);
5. the pH of the binder liquid, ranging from 2.2 to 5.4 (formulations 14–17 and 11).

A scale of five values for each variable with a constant level of the other variables at the centre value of the experimental design was chosen.

Twenty-nine formulations were employed to perform a statistical experimental plan based on the centre of gravity design, as outlined by Podczeck (1995), see Table 1. The basic design was expanded to evaluate the effect of the pH of the distilled water instead of the buffer, formulation 10; the interaction between the minimum pH value (pH 2.2) and the 4% level of each added polymer, formulations 20 and 21; the interaction between the maximum pH value (pH 5.4) and the 4% level of each added polymer, formulations 22 and 23; the equilibrium time between preparation and processing of 0, 18 and 36 h, formulations 24, 11 and 25. In these formulations the quantity of lactose varies. It was not considered as a variable because it represents the missing amount of additive necessary to ensure the total solid content was 100%.

The influence of these variables, if any, on the required amount of liquid binder, the steady-state extrusion force, the characteristics of the pellets in terms of size, and its distribution, shape, density and porosity plus the in vitro drug release by dissolution testing was the objective of the work.

Table 1
The experimental plan used to investigate the influence of chitosan, sodium alginate and formulation variables on the formation and drug release from pellets

Formulation	LP (%)	LA (%)	LC (%)	LSA (%)	LL (%)	PH
1	2.5	50	2	2	43.5	3.8
2	5	50	2	2	41	3.8
3	20	50	2	2	26	3.8
4	40	50	2	2	6	3.8
5	10	30	2	2	56	3.8
6	10	40	2	2	46	3.8
7	10	60	2	2	26	3.8
8	10	70	2	2	16	3.8
9	10	50	4	0	36	3.8
10	10	50	3	1	36	3.8
11[a]	10	50	2	2	36	3.8
12	10	50	1	3	36	3.8
13	10	50	0	4	36	2.2
14	10	50	2	2	36	3.0
15	10	50	2	2	36	4.6
16	10	50	2	2	36	5.4
17	10	50	2	2	36	5.2
18	10	50	2	2	36	3.8
19	10	50	0	0	40	2.2
20	10	50	4	0	36	2.2
21	10	50	0	4	36	5.4
22	10	50	4	0	36	5.4
23	10	50	0	4	36	3.8
24[b]	10	50	2	2	36	3.8
25[c]	10	50	2	2	36	3.8
26	10	50	8	0	32	3.8
27	10	50	16	0	24	3.8
28	10	50	0	8	32	3.8
29	10	50	0	16	24	3.8

LP: level of paracetamol; LA: level of microcrystalline cellulose (Avicel PH101); LC: level of chitosan; LSA: level of sodium alginate; LL: level of lactose; PH: pH level of binder liquid.
[a] Centre of gravity experiment with the equilibrium time of 18 h.
[b] Equilibrium time of 0 h.
[c] Equilibrium time of 36 h.

3. Results and discussion

3.1. Initial assessment

3.1.1. Binder liquid level

The "best" binder liquid level (BL) required for wetting each formulation of the dry mixtures was chosen, based on the quality of the pellets in terms of the uniformity of size and sphericity data. This level of binder liquid produced the pellets in the most frequently occurring sieve fraction (1400–1700 μm) with the highest value for roundness assessment. The total yield of pellets was not considered as a criterion in this study, as the differences were found to be relatively small with changes in a range of binder liquid levels studied. The values of BL (percentage based on the total dry powder weight) appeared to range from 40 to 74.8%, depending on the formulation variables, see Table 2. The minimum value of BL was required for formulation no. 5 containing 30% MCC, and formulations no. 13 and no. 23 containing 4% sodium alginate, while the maximum value of BL was required for formulation no. 27 containing 16% chitosan. This suggests that the value of BL was likely to be dependent on the values of LA, LSA and LC. In the group of experiments, which varied the value of LA, it can

Table 2
Extrusion force (mean and standard deviation, $n = 4$) and the binder level for the best formulations used to investigate the influence of chitosan, sodium alginate and formulation variables on the formation and drug release from pellets

Formulation	Extrusion force (kN)	Binder liquid level (%)
1	11.83 (0.28)	43.2
2	10.97 (0.11)	43.2
3	9.61 (0.11)	44.8
4	9.06 (0.14)	48.8
5	7.75 (0.06)	40.0
6	9.50 (0.06)	42.0
7	12.00 (0.09)	46.0
8	10.58 (0.11)	56.8
9	6.19 (0.14)	56.0
10	6.39 (0.11)	54.0
11	10.42 (0.17)	45.0
12	6.58 (0.11)	50.0
13	11.83 (0.45)	40.0
14	9.97 (0.11)	44.8
15	9.67 (0.09)	44.8
16	10.19 (0.06)	44.8
17	10.08 (0.06)	44.8
18	7.72 (0.33)	45.0
19	5.78 (0.00)	54.0
20	6.69 (0.19)	60.0
21	10.75 (0.14)	47.2
22	6.14 (0.14)	54.0
23	9.92 (0.28)	40.0
24	9.58 (0.14)	47.2
24[a]	10.53 (0.06)	47.2
25	11.28 (0.11)	45.0
26	4.11 (0.13)	70.0
27	4.61 (0.06)	74.8
28	6.94 (0.11)	48.0
29	4.19 (0.06)	56.0

SS: steady state.
[a] Formulation no. 24, the binder liquid level of which was identical to that of formulations no. 11 and no. 25 but 24 extruded immediately.

be seen that the value of BL gradually increased from 40 to 46% when the value of MCC increased from 30 to 60%, and then a sudden increase in the value of BL occurred up to 56.8%, with a value of LA of 70% (Fig. 1). This can be explained by the "molecular sponge" structure of MCC in which it is possible to retain a high amount of water, as described by Fielden et al. (1988). Therefore, the higher the value of LA in the formulation, the higher the quantity of the binder liquid required to form a suitable consistency of the wet powder mass.

The influence of the ratio of LC to LSA on the value of BL was not so clear at this stage. In fact, a relatively high value of LC required a higher level of binder liquid for the "best" pellet formulation (Fig. 2). Likewise, when only chitosan was incorporated at 4, 8 and 16% levels in formulations no. 9, no. 26 and no. 27, the values of BL of 56, 70 and 74.8%, respectively, were considerably higher than the BL value of 54% required for formulation no. 19 containing no added polymer. This effect indicates that chitosan may possess the ability to absorb water in a similar manner to MCC. No marked change in the value of BL was observed when other formulation factors, such as the pH level and the type of binder liquid, were varied, although there was a slight increase in the value of BL when the amount of paracetamol was increased.

To obtain the "best" pellet formulation, formulation no. 24, which was extruded immediately after the preparation of the wet powder mass, required a slightly higher level of binder liquid than that for formulations no. 11 and no. 25, which had the same formulation variables but were equilibrated before the extrusion process. This may be due to the equilibrium time allowing the binder liquid to distribute throughout the wet powder mass. Therefore, a good consistency of the wet powder mass, and hence of the extrudate, could be obtained at a relatively lower level of binder liquid, if equilibrium is allowed to occur.

3.1.2. Extrusion force

The steady-state extrusion forces occurred in the force/displacement profiles of the extrusion processes for all the formulations studied, with no forced flow stage. This information indicated that the uniformity of the extrudate throughout the process of extrusion was obtained, with no water migration as extrusion proceeds.

The steady-state force (SSF) required for the "best" formulation varied from 4.11 to 12.00 kN (Table 2). The former value of SSF was required for the extrusion of formulation no. 26 containing 8% chitosan, while the latter value of SSF was required for the extrusion of formulation no. 7 containing 60% MCC. The influence of each studied factor was however, not so clear. The wide range of SSF values could be explained by the characteristics of the wet powder mass together with the formulation, which contained a higher level of binder liquid, providing more lubrication to the wet

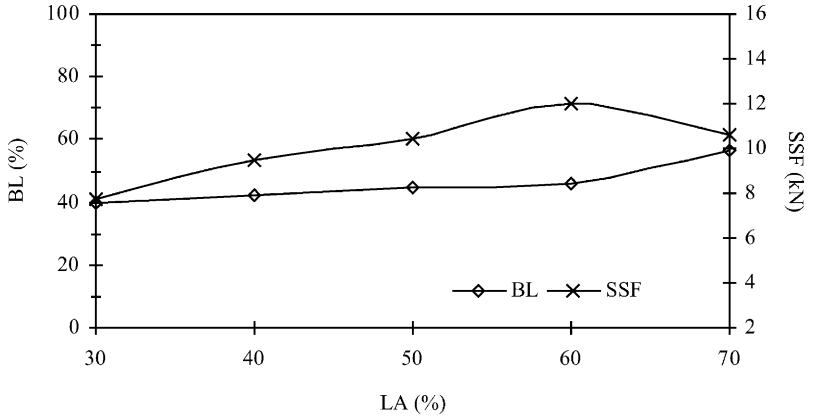

Fig. 1. The influence of the levels of microcrystalline cellulose (LA) on the "best" binder liquid levels (BL) and the steady-state extrusion forces required for the "best" formulations (SSF).

powder mass during the extrusion process. For example, the lower SSF value required for the extrusion of the wet powder mass with the higher level of BL, when the ratio of the LC to the LSA was varied (Fig. 2).

It was also observed that a slight change in the value of SSF occurred when the same composition of the dry mixtures (formulations no. 24, no. 11, no. 25) were wetted with an identical water level, then immediately extruded or equilibrated for different periods of time (Table 2). Compared to the formulation extruded immediately after preparing the wet powder mass, a gradual decrease, from 10.53 to 10.42 kN, in the value of SSF when the wet powder mass was equilibrated for 18 h could be caused by the effect of the distribution of binder liquid throughout the wet powder mass, which could soften the mass, whereas the possibility of losing the moisture of the wet mass when the equilibrium period was prolonged to 36 h could explain the return of the higher SSF value of 11.28 kN. There was a slight change in the value of SSF when the pH of the buffer was varied, but a marked decrease, from 10.42 kN of formulation no. 11 to 7.72 kN of formulation no. 18, in the value of SSF can be seen when the same weight of distilled water was used as the binder liquid instead of the buffer.

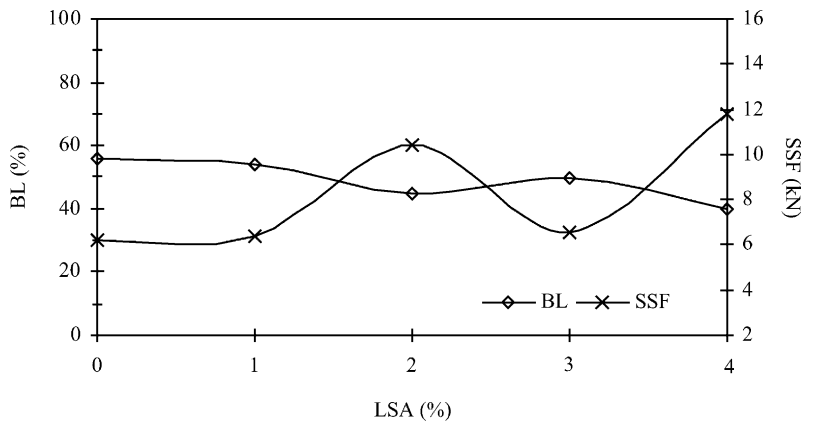

Fig. 2. The influence of the ratios of chitosan (LC) to sodium alginate (LSA) with a total level of 4%, represented by the values of LSA, on the "best" binder liquid levels (BL) and the steady-state extrusion forces required for the "best" formulations (SSF).

Table 3
Weight in modal sieve fractions, median diameter (d_g) and inter-quartile range (IQR) of pellets produced from the best formulations used to investigate the influence of chitosan, sodium alginate and formulation variables on the formation and drug release from pellets

Formulation	Sieve fraction (μm)	Weight (%) in modal sieve fraction	d_g (μm)	IQR (μm)
1	1700–2000	42.84	1698	108
2	1400–1700	49.55	1652	108
3	1700–2000	46.89	1675	107
4	1400–1700	49.75	1645	107
5	1700–2000	47.72	1694	106
6	1700–2000	46.12	1683	107
7	1400–1700	60.85	1645	107
8	1700–2000	44.95	1653	108
9	1000–1400	80.50	1293	106
10	1400–1700	57.04	1584	107
11	1400–1700	49.33	1664	108
12	1700–2000	43.30	1702	108
13	1400–1700	62.25	1594	108
14	1400–1700	51.14	1629	108
15	1400–1700	45.27	1658	107
16	1700–2000	51.62	1695	107
17	1400–1700	47.15	1657	108
18	1400–1700	46.62	1693	110
19	1000–1400	54.09	1383	105
20	1000–1400	66.56	1339	106
21	1400–1700	62.46	1576	108
22	1000–1400	82.90	1296	105
23	1400–1700	43.90	1707	108
24	1400–1700	47.73	1665	108
24[a]	1400–1700	49.95	1662	108
25	1400–1700	49.18	1658	108
26	1400–1700	53.33	1406	106
27	1000–1400	79.60	1284	107
28	1700–2000	47.24	1711	108
29	1400–1700	33.79	2017	120

[a] Formulation no. 24, the binder liquid level of which was identical to that of formulations no. 11 and no. 25 but 24 extruded immediately.

3.1.3. Characterisation of the pellets

3.1.3.1. Size analysis. The results of sieve analysis showed that for the formulations studied, the most frequently occurring sieve fraction was 1400–1700 μm, see Table 3. Thus, this size range was used for the evaluation. In relation to sieve fraction 1000–1400 μm, the yield of pellets in sieve fraction 1400–1700 μm was generally higher, except for formulations no. 9, no. 20, no. 22 and no. 27 containing chitosan and without sodium alginate and formulation no. 19 with no chitosan and sodium alginate.

The median diameter of pellets (d_g) varied from 1284 μm (formulation no. 27 containing 16% chitosan) to 2017 μm (formulation no. 29 containing 16% sodium alginate), depending on the formulation variables. The inter-quartile range (IQR) values were approximately 100 μm, indicating a very narrow size range of pellets was produced. In fact, with a relatively small difference in the size distribution, it can be observed that the highest value of the IQR was 120 μm and was obtained from formulation no. 29 containing 16% sodium alginate. The lowest value of the IQR was 102 μm, observed with formulation no. 19 containing no added polymer and no. 22 containing 4% chitosan. For example, nearly 90% of pellets lying within two consecutively selected sieve fractions (1000–1400 and 1400–1700 μm) were produced from formulations no. 9, no. 20, no. 22, no. 26, and no. 27, which contained only chitosan and formulation no. 19 with no added polymer. Thus, overall results reflected that the size and size distribution of pellets were influenced by the presence of chitosan and/or sodium

alginate in the formulation. The smaller size and uniform size distribution, in particular within the selected size range (1000–1700 μm) could be obtained when only chitosan was incorporated into the formulation or there was no added polymer in the formulation. This implied that the extrudate containing sodium alginate could not be easily broken into short lengths and possessed lower plasticity than the extrudate, which did not contain sodium alginate. The spheronisation time of 30 min required for all formulations, which contained sodium alginate confirmed this conclusion.

Other formulation factors studied, including the values of LP, LA, PH, and the equilibrium time exerted slight influence on the size of pellets produced from the "best" formulation. There was no relationship between the value of BL, which was required for each formulation and the size of pellets produced.

3.1.3.2. Shape and shape outline. The values of the shape factor, which varied from 0.5211 to 0.6799 and the aspect ratio ranging from 1.058 to 1.144, represented the best roundness of pellets (Table 4). A maximum value of the shape factor and a minimum value of the aspect ratio was obtained for formulation no. 18 in which each polymer was incorporated at 2% level and distilled water was used as the binder liquid, while a minimum value of the shape factor and a maximum value of the aspect ratio was observed in formulation no. 22 containing 4% of chitosan and using the pH 5.4 buffer as the binder liquid. With respect to the pH level of binder liquid, there were differences in the values of shape parameters, yet the relation between the pH level of binder liquid and the value of shape parameters cannot be identified. On the other hand, changes in the shape of pellets can be related to the value of

Table 4
Shape factor, aspect ratio and perimeter (mean and standard deviation, $n = 30$–40) of pellets, in sieve fraction 1400–1700 μm, produced from the formulations used to investigate the influence of chitosan, sodium alginate and formulation variables on the formation and drug release from pellets

Formulation	Binder liquid level (%)	Shape factor	Aspect ratio	Perimeter (μm)
1	43.2	0.5858 (0.0888)	1.101 (0.045)	5489 (256.3)
2	43.2	0.5849 (0.1196)	1.134 (0.233)	5342 (325.9)
3	44.8	0.6530 (0.1095)	1.072 (0.046)	5199 (256.6)
4	48.8	0.5908 (0.1128)	1.105 (0.069)	5280 (252.2)
5	40.0	0.6125 (0.0887)	1.088 (0.040)	5408 (262.8)
6	42.0	0.6559 (0.0952)	1.070 (0.044)	5266 (310.0)
7	46.0	0.5754 (0.1215)	1.116 (0.069)	5615 (275.8)
8	56.8	0.6240 (0.1103)	1.087 (0.057)	5410 (209.1)
9	56.0	0.5591 (0.0850)	1.116 (0.045)	4849 (120.9)
10	54.0	0.6262 (0.0771)	1.080 (0.036)	5160 (224.0)
11	45.0	0.6399 (0.1247)	1.101 (0.195)	5161 (332.3)
12	50.0	0.6394 (0.0821)	1.087 (0.084)	5229 (975.7)
13	40.0	0.6017 (0.1069)	1.096 (0.050)	5298 (257.7)
14	44.8	0.5865 (0.1123)	1.107 (0.060)	5515 (272.5)
15	44.8	0.6247 (0.1009)	1.085 (0.052)	5300 (220.8)
16	44.8	0.6113 (0.0985)	1.088 (0.048)	5175 (253.7)
17	44.8	0.6249 (0.0995)	1.080 (0.041)	5100 (296.6)
18	45.0	0.6799 (0.1055)	1.058 (0.045)	5087 (261.6)
19	54.0	0.5915 (0.1038)	1.104 (0.074)	4888 (248.8)
20	60.0	0.6278 (0.1165)	1.095 (0.117)	4585 (205.4)
21	42.4	0.5504 (0.1269)	1.134 (0.079)	5769 (314.4)
22	54.0	0.5211 (0.0951)	1.144 (0.057)	5218 (153.7)
23	40.0	0.5879 (0.1356)	1.117 (0.100)	5421 (230.6)
24	47.2	0.6429 (0.1049)	1.075 (0.043)	5342 (245.8)
25	45.0	0.6156 (0.1377)	1.131 (0.280)	5459 (379.7)
26	70.0	0.6121 (0.0702)	1.089 (0.037)	4699 (222.9)
27	74.8	0.5928 (0.0984)	1.095 (0.048)	5550 (129.4)
28	48.0	0.5782 (0.1241)	1.118 (0.077)	5563 (238.9)
29	56.0	0.5315 (0.1043)	1.139 (0.063)	5990 (401.6)

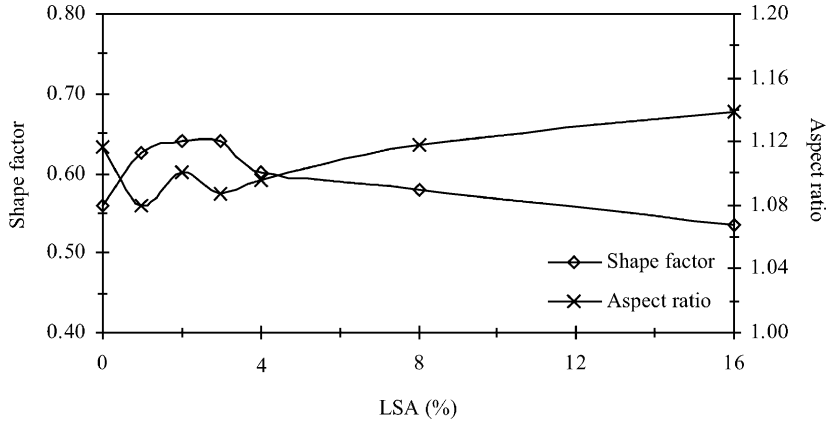

Fig. 3. The influence of the levels of sodium alginate (LSA), with the presence and absence of chitosan in the formulation, on the shape of pellets for the "best" formulations.

LSA and/or the value of LC in the formulations. It was observed that from 1 to 3% of the LSA (Fig. 3), the presence of both sodium alginate and chitosan in the formulations improved the roundness of pellets, reflecting an increase in the value of the shape factor. The value of the shape factor slightly decreased when sodium alginate or chitosan was incorporated alone at 4, 8 and 16% levels. Although the aspect ratio of pellets was generally congruent with the shape factor, the aspect ratio was a poor indicator of the difference between each formulation and hence it will not be discussed further here. It is clear that the value of the aspect ratio is not a sensitive parameter to assess the sphere quality in spite of its persistent use in studies of pellet properties.

The perimeter of pellets was proposed to be a parameter defining the size of pellets in sieve fraction 1400–1700 μm. Except for formulations no. 9, no. 20 and no. 26 in which only chitosan was added, and formulation no. 19 with no added polymer, having perimeter values of less than 5000 μm, most of the formulations had perimeter values of greater than 5000 μm. The minimum perimeter value (4585 μm) was found for formulation no. 20 containing 4% chitosan, while the maximum perimeter value (5990 μm) was obtained for formulation no. 29 with 16% sodium alginate. Therefore, these results could support the influence of chitosan and sodium alginate on the pellet size, as previously discussed.

3.1.3.3. Density and porosity. The density of pellets produced from most of the formulations studied appeared to be in the range of 1300–1400 kg/m^3 (Table 5). The higher density of pellets was found for formulation no. 5 (1437 kg/m^3) containing 30% MCC, formulations no. 21 (1418 kg/m^3) and no. 29 (1488 kg/m^3) which contained 4 and 16% sodium alginate, respectively, while the lower density of pellets was obtained from formulation no. 27 (1281 kg/m^3) with 16% chitosan.

In the group, which varied the value of LA, a lower density of pellets was observed when the level of MCC in the formulation was increased from a 30% level. Further, looking at the influence of chitosan or sodium alginate incorporated alone at 4, 8 and 16% levels in the formulations, there was a relatively marked decrease and increase in the density of pellets only at 16% values of LC and of LSA, respectively. The change in the density of pellets cannot be clearly related to the density of MCC (1558 kg/m^3), chitosan (1472 kg/m^3) and sodium alginate (1702 kg/m^3).

The porosity (here defined as 1—the ratio of the apparent pellet density to the apparent particle density) of pellets produced was generally higher than 10%, although the values varied from 0.042 (4.2%) to 0.155 (15.5%) (Table 5). A minimum porosity of pellets was observed for formulation no. 29 containing 16% sodium alginate, while a maximum porosity was found for formulation no. 27 containing 16%

Table 5
Density and porosity (mean and standard deviation, $n = 3$) of pellets, in sieve fraction 1400–1700 μm, produced from the best formulations used to investigate the influence of chitosan, sodium alginate and formulation variables on the formation and drug release from pellets

Formulation	Density (kg/m^3)	Porosity
1	1340 (0.001)	0.135 (0.001)
2	1339 (0.001)	0.132 (0.001)
3	1325 (0.001)	0.121 (0.000)
4	1324 (0.001)	0.092 (0.000)
5	1437 (0.002)	0.060 (0.001)
6	1344 (0.001)	0.122 (0.001)
7	1332 (0.001)	0.131 (0.001)
8	1325 (0.000)	0.135 (0.000)
9	1340 (0.001)	0.122 (0.001)
10	1334 (0.001)	0.127 (0.000)
11	1319 (0.000)	0.138 (0.000)
12	1299 (0.000)	0.153 (0.000)
13	1348 (0.001)	0.122 (0.001)
14	1324 (0.001)	0.135 (0.000)
15	1343 (0.001)	0.122 (0.000)
16	1336 (0.001)	0.127 (0.001)
17	1366 (0.001)	0.108 (0.001)
18	1342 (0.001)	0.123 (0.001)
19	1385 (0.000)	0.095 (0.000)
20	1372 (0.001)	0.101 (0.000)
21	1418 (0.001)	0.076 (0.000)
22	1359 (0.002)	0.110 (0.001)
23	1332 (0.002)	0.133 (0.001)
24	1304 (0.001)	0.148 (0.001)
24[a]	1322 (0.001)	0.137 (0.001)
25	1338 (0.001)	0.126 (0.001)
26	1310 (0.001)	0.140 (0.001)
27	1281 (0.001)	0.155 (0.000)
28	1356 (0.001)	0.120 (0.001)
29	1488 (0.001)	0.042 (0.001)

[a] Formulation no. 24, the binder liquid level of which was identical to that of formulations no. 11 and no. 25 but 24 extruded immediately.

chitosan. This difference reflected that a dense structure of pellets possibly resulted from the addition of sodium alginate and a looser structure of pellets could be obtained from the presence of chitosan in the formulation. However, such conclusion cannot be easily established, due to the fact that the results involved a number of formulation variables. It was observed that the higher the density of pellets, the lower the porosity. The influence of the added polymers on the density and porosity of pellets is shown in Fig. 4.

3.1.4. In vitro drug release

Disintegration was observed to occur within the first 5–10 min of the dissolution time for the pellets produced from formulations no. 9, no. 20, no. 22, no. 26 and no. 27, which contained varying amounts of chitosan and with no sodium alginate. In each case, the value of LC did not appear to be an important factor for the disintegration time of the pellets. The reason of this disintegrating effect was not identified, although it could be attributed to the property of chitosan acting as a disintegrant, as has been used in a tablet formulation (e.g. Sawayanagi et al., 1982). Conversely, the addition of chitosan, had previously reported to retard drug release (e.g. Tapia et al., 1993; Goskonda and Upadrashta, 1993). The pellets produced from other formulations, except formulation no. 10 with 3% of chitosan and 1% of sodium alginate, which had extended disintegration times, remained intact after completion of the dissolution studies. Typical dissolution profiles of some formulations used to investigate the influence of the formulation variables are illustrated in Fig. 5.

The characteristics of the dissolution profiles, assessed by the area under the dissolution curve (AUC), the mean dissolution time (MDT) and the variance associated with the MDT (VR) of drug (paracetamol) release from pellets, are presented in Table 6. The values of VR were found to be 0.00 h^2 for formulations no. 9, no. 20, no. 22, no. 26 and no. 27. The steepness of the curve was attributed to the disintegration of pellets made from these formulations. The lower values of VR were also found for the pellets with faster drug release. From Fig. 6, it is apparent that when the ratio of sodium alginate to chitosan increased, with a total level of chitosan and sodium alginate of 4%, the values of AUC and MDT increased. The substitution of chitosan by 1% of sodium alginate resulted in a reduction of the disintegration effect of chitosan, and was reflected by an extended disintegration time. No disintegration effect was observed when incorporating equal parts of chitosan and sodium alginate or when the relatively high levels of LSA were studied. In relation to formulation no. 19 with no added polymer (AUC = 23.9% h; MDT = 0.25 h), an increase in the value of AUC from 5.8 to 36.8% h and in the value of MDT from 0.06 to 0.38 h occurred when the level of sodium alginate increased from 0 to 4% in formulations no. 9–13. This could indicate a relatively

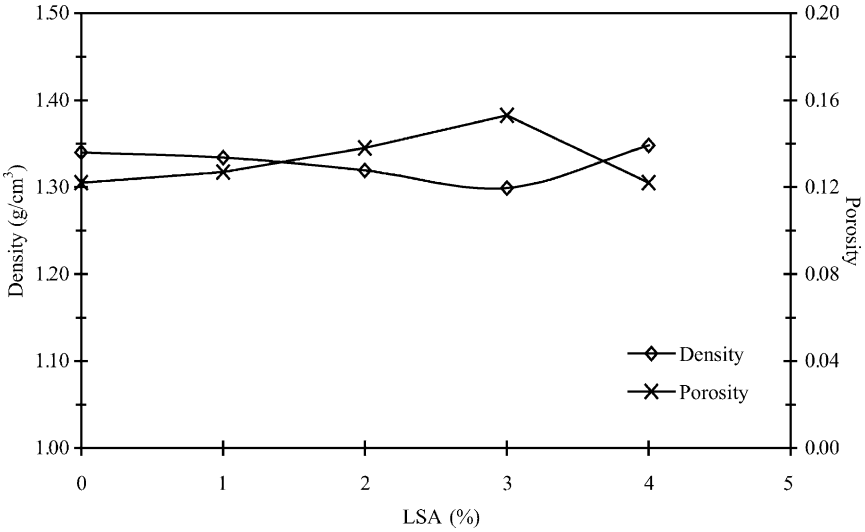

Fig. 4. The influence of the ratios of chitosan (LC) to sodium alginate (LSA) with a total level of 4%, represented by the values of LSA, on the density and porosity of pellets for the "best" formulations.

prolonged drug release effect of sodium alginate in the pellets. However, the values of AUC and MDT for formulations no. 28 (AUC = 27.7% h; MDT = 0.29 h) and no. 29 (AUC = 34.0% h; MDT = 0.35 h) did not show a greater effect by increasing the value of LSA up to 8 and 16%, respectively.

In addition to the influence of chitosan and sodium alginate in the formulations, changes in the values of AUC and MDT were observed when the level of LP was varied (Fig. 7). Increasing the level of LP in pellets from 2.5 to 40% resulted in an increase in the value of AUC from 16.8 to 71% h and an increase in the value of MDT from 0.18 to 0.75 h. This may be due to the change in the porosity of pellets from 0.135 (13.5%) to 0.092 (9%) and the corresponding reduction of lactose content (LL) from 43.5 to 6% when the value of LP in the formulation was increased. Lactose is a highly soluble excipient in the formulation. Therefore, during the dissolution process there was the possibility of a rapid increase in the porosity of pellets, which contained considerable amounts of lactose. These pellets allow the dissolution medium to penetrate into the

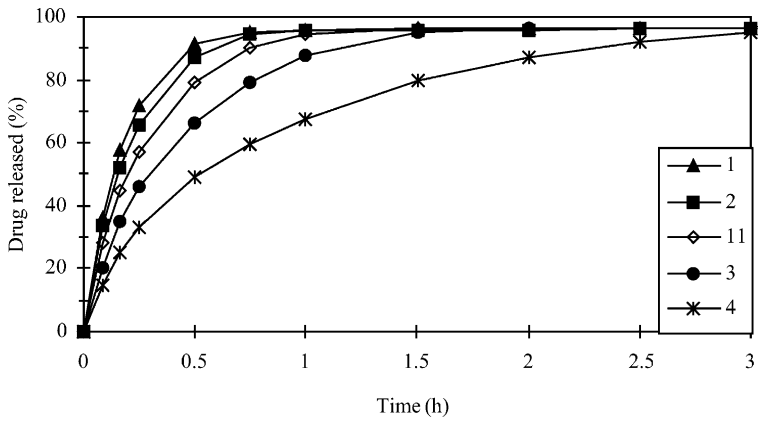

Fig. 5. The influence of the levels of paracetamol (LP): (1) 2.5%, (2) 5%, (11) 10%, (3) 20%, (4) 40%, on drug (paracetamol) release from pellets.

Table 6
Area under the dissolution curve, AUC; mean dissolution time, MDT; variance associated with the MDT, VR; relative dispersion of the dissolution time, RD (mean and standard deviation, $n = 6$) and release model, RM of drug release from pellets, in sieve fraction 1400–1700 μm, produced from the best formulations used to investigate the influence of chitosan, sodium alginate and formulation variables on the formation and drug release from pellets

Formulation	AUC (% h)	MDT (h)	VR (h^2)	RD	RM[a]
1	16.8 (0.5)	0.18 (0.01)	0.03 (0.01)	0.774 (0.017)	2
2	20.1 (0.4)	0.21 (0.00)	0.04 (0.01)	0.817 (0.020)	2
3	37.6 (0.9)	0.39 (0.01)	0.14 (0.03)	0.828 (0.014)	2
4	71.0 (1.5)	0.75 (0.02)	0.53 (0.03)	0.952 (0.010)	1
5	22.0 (0.4)	0.23 (0.01)	0.04 (0.00)	0.667 (0.013)	3
6	23.8 (0.6)	0.24 (0.01)	0.05 (0.00)	0.821 (0.009)	2
7	25.6 (0.6)	0.27 (0.01)	0.05 (0.00)	0.704 (0.006)	N
8	34.9 (0.9)	0.37 (0.01)	0.13 (0.01)	0.944 (0.021)	1
9	5.8 (0.2)	0.06 (0.00)	0.00 (0.00)	0.372 (0.012)	0
10	15.1 (0.3)	0.16 (0.00)	0.01 (0.00)	0.508 (0.008)	N
11	25.0 (0.3)	0.26 (0.01)	0.06 (0.01)	0.790 (0.008)	2
12	27.5 (0.5)	0.29 (0.00)	0.06 (0.00)	0.764 (0.021)	2
13	36.8 (0.8)	0.38 (0.01)	0.13 (0.01)	0.930 (0.033)	1
14	27.9 (0.3)	0.29 (0.00)	0.07 (0.00)	0.810 (0.026)	2
15	23.6 (0.9)	0.25 (0.01)	0.05 (0.01)	0.785 (0.015)	2
16	28.5 (0.6)	0.30 (0.01)	0.08 (0.01)	0.897 (0.037)	N
17	26.1 (0.9)	0.27 (0.01)	0.06 (0.01)	0.755 (0.020)	2
18	21.9 (0.4)	0.23 (0.00)	0.04 (0.01)	0.652 (0.007)	3
19	23.9 (0.3)	0.25 (0.01)	0.05 (0.00)	0.826 (0.021)	2
20	8.6 (0.2)	0.09 (0.00)	0.00 (0.00)	0.303 (0.011)	0
21	35.9 (0.2)	0.37 (0.00)	0.13 (0.01)	0.907 (0.007)	N
22	7.2 (0.2)	0.08 (0.01)	0.00 (0.00)	0.335 (0.012)	0
23	37.2 (0.6)	0.39 (0.01)	0.13 (0.01)	0.841 (0.009)	2
24	26.0 (0.5)	0.27 (0.01)	0.05 (0.00)	0.721 (0.016)	N
25	25.9 (0.8)	0.27 (0.01)	0.05 (0.01)	0.738 (0.011)	N
26	4.7 (0.3)	0.05 (0.00)	0.00 (0.00)	0.262 (0.042)	0
27	4.2 (0.2)	0.05 (0.01)	0.00 (0.00)	0.154 (0.081)	N
28	27.7 (1.1)	0.29 (0.01)	0.07 (0.00)	0.795 (0.031)	2
29	34.0 (1.0)	0.35 (0.01)	0.11 (0.01)	0.901 (0.005)	N

[a] Release model: 1, first order; 2, square root; 3, cube root; 0, zero order; N, no simple kinetics.

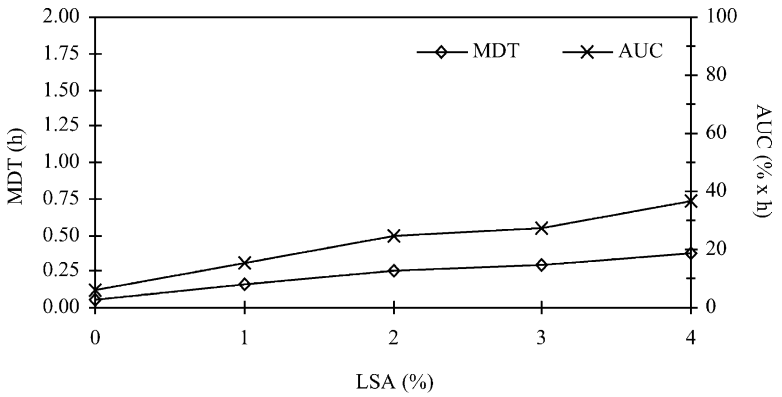

Fig. 6. The influence of the ratios of chitosan (LC) to sodium alginate (LSA) with a total level of 4%, represented by the values of LSA, on the mean dissolution time (MDT) and the area under the dissolution curve (AUC) of pellets.

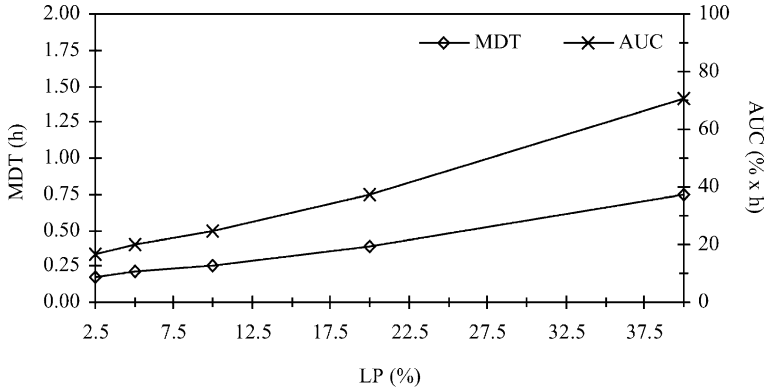

Fig. 7. The influence of the levels of paracetamol (LP) on the mean dissolution time (MDT) and the area under the dissolution curve (AUC) of pellets.

matrix quickly and hence ensure fast release of paracetamol.

In the set of the formulations in which the level of LSA was the variable, an increase in the values of AUC and MDT of drug release can be seen for formulation no. 8 with 70% MCC (AUC = 34.9% h, MDT = 0.37 h) (Fig. 6). This effect may also be explained by the influence of lactose content (16%), which was relatively small in this formulation and may be due to the matrix behaviour of MCC with a substantial amount in the formulation, which could prolong the drug release. The influence of other formulation factors on the values of AUC and MDT of drug release was not readily apparent. Nevertheless, it is possible that the dissolution behaviour of most pellet formulations could be dominated by a level of lactose of at least 36% in the pellet, as has been reported previously (Blanque et al., 1995).

The mechanism of drug release from pellets could be identified, based on the value of the relative dispersion of dissolution time (RD), as proposed by Voegele et al. (1988). A compromise must be made, based on the value of standard deviation, in order to fit the kinetics of drug release into one of the mechanism models (Table 6). In fact, it was apparent that the drug release for some formulations of pellets studied did not follow the defined release mechanisms, or overlapping release mechanisms may occur.

For most of the formulations studied, the release rate mechanisms were dominated by the diffusion process (approximate RD = 0.8), in which the dissolution medium penetrated into the pore of the matrix and dissolved paracetamol, which then diffused out from the pellet. The influence of the level of LP on the release rate mechanism of paracetamol was evident. Here formulation no. 4 with a maximum paracetamol level (40%) was indicated as a first order mechanism (RD = 0.952 ± 0.010). Thus, paracetamol release from pellets was dependent on the amount of paracetamol remaining in the pellets. The release rate mechanism of paracetamol from pellets of formulation no. 8 containing a maximum level of MCC (70%) was the same (RD = 0.944±0.021). The dissolution of paracetamol seemed to be the rate-limiting factor (RD = 0.667 ± 0.013) for its release from pellets of formulation no. 5 with the minimum level of MCC (30%). This was also likely to control the release rate of paracetamol from pellets of formulation no. 18, where distilled water was the binder liquid (RD = 0.652 ± 0.007).

Further, a marked influence on the release rate mechanism can be related to the ratio of chitosan to sodium alginate. When the ratio of LC to LSA was changed from 4:0, 2:2, 1:3 to 0:4, the release rate mechanism changed from zero order (RD = 0.372 ± 0.012), square root (RD = 0.790 ± 0.008), square root (RD = 0.764 ± 0.021) and became first order mechanism (RD = 0.930 ± 0.033). At a 3:1 ratio of LC to LSA, the mechanism of paracetamol release cannot be identified with simple kinetics (RD = 0.508 ± 0.008). Thus, it was apparent that the first order mechanism was present for paracetamol release from pellets of the formulations containing

sodium alginate as a single added polymer, while the zero order mechanism of paracetamol release can be seen for the formulations, which contained chitosan as a single added polymer and where the disintegration effect was observed. However, due to the disintegration effect within the first measuring time, the RD values of the latter formulations were the estimated values and hence the zero order mechanism could not be validated. In addition, the interpretation of the results could be influenced by a quantity of lactose in the formulations as described earlier.

3.2. Statistical analysis

Three procedures of statistical analysis were applied in an attempt to identify a relationship between formulation variables and formulation responses.

The analysis of variance was first applied to screen whether there was a significant difference among the sample means of data for each factor studied, hence the non-significant parameters could be excluded from the subsequent statistical analyses. Second, the canonical analysis, which is a multivariate analysis, was conducted to signify the influence of each single parameter and the interrelationship between two groups of variables. Finally, as suggested by the first and the second statistical analysis, multiple regression analysis was employed to identify possible empirical models of the relationship between significant formulation variables and formulation behaviours.

3.2.1. Analysis of variance

The results of the analysis of variance are shown in Table 7. Since for each formulation, the data of size analysis were obtained from one experiment, they cannot be compared using this statistical analysis. At a significance level (P) of 0.05, there was a significant difference in the values of SSF, perimeter, density and porosity of pellets, responding to the change of each factor studied. No significant difference can be seen in the value of the aspect ratio of any of the groups, although there was a significant difference reflected in the value of the shape factor. A significant difference in the shape factor of pellets was not, however, present when the equilibrium time of the wet mass was varied or when the interaction between the levels of PH and LSA in the formulation was evaluated. Nevertheless, as the sphericity data were the criteria for selecting the "best" binder liquid, it is reasonable that the values of the shape factor and the aspect ratio of the pellets produced from the "best" formulations were very similar.

The values of AUC and MDT of the drug release profiles varying, with significant differences and were influenced by each factor studied. There was no significant difference in the value of MDT when varying the equilibrium time before extrusion for the "best" pellet formulation.

3.2.2. Canonical analysis

The canonical analysis as applied by Podczeck et al. (1993) to a pharmaceutical study was employed

Table 7
The results of the analysis of variance (F-values) comparing the sample means of data for each factor studied

Group[a]	SSF[b]	Shape factor[b]	Aspect ratio[b]	Perimeter[b]	Density[b]	Porosity[b]	AUC[b]	MDT[b]
I	**158.48**	**2.82**	1.00	**6.67**	**353.75**	**2644.17**	**4112.10**	**2334.44**
II	**872.50**	**2.48**	1.00	**12.17**	**4671.25**	**5288.93**	**425.10**	**187.80**
III	**839.19**	**3.93**	1.48	**34.26**	**>17724.44**	**6533.53**	**2525.84**	**1571.67**
IV	**142.18**	**2.61**	1.04	**10.64**	**871.20**	**689.31**	**99.14**	**60.00**
V	**29.76**	**6.72**	2.39	**63.44**	**662.48**	**829.36**	**8119.13**	**920.04**
VI	**372.48**	1.18	1.49	**60.03**	**2669.71**	**4016.52**	**867.43**	**343.30**
VII	**143.01**	1.00	1.00	**7.07**	**1827.00**	**546.87**	**5.57**	2.00
VIII	**58.94**	1.04	1.00	**7.75**	**656.00**	**199.08**	–	–

SSF: steady-state extrusion force for the "best formulation; AUC: the area under dissolution curve; MDT: the mean dissolution time" (–) not studied.

[a] I, formulations no. 1–4 and no. 11; II, formulations no. 5–8 and no. 11; III, formulations no. 9–13, no. 19, and no. 26–29; IV, formulations no. 14–18 and no. 11; V, formulations no. 9, no. 19, no. 20, and no. 22; VI, formulations no. 13, no. 19, no. 21, and no. 23; VII, formulations no. 11, no. 24 and no. 25; VIII, formulations no. 11, no. 24, and no. 25.

[b] Bold characters show significant differences at a significance level (P) of 0.05.

to qualify and quantify the interrelationship between two groups of variables which were the influencing factors or independent variables, *X*, and the dependent variables, *Y*. The basic requirement for the applicability of the canonical analysis, such as a number of experiments performed with replications and significant difference of values included in the group of independent variables which were unrelated to each other, must be achieved.

In the present analysis, based on the result of the analysis of variance, the independent variables were the formulation variables, including the values of LP, LA, LC, LSA and pH, and dependent variables were the formulation responses, including the values of BL, SSF, the performance of pellets (size (d_g), shape factor, perimeter, density, porosity), and the characteristics of dissolution profiles of paracetamol pellets (AUC and MDT). The aspect ratio was not considered here as ANOVA had identified this parameter as non-significant. The canonical analysis was divided into three sections. The first section considered the influence of formulation variables on the values of BL, SSF and on the perimeter, density and porosity of pellets; and the second section considered the influence of formulation variables on the shape factor and the median diameter values of pellets. These two sections were proposed to evaluate the influence of the formulation variables on the pellet formation relating to the extrusion and spheronisation process. Finally, the third section considered the influence of formulation variables on the characteristics, in terms of the values of AUC and MDT, of the in vitro dissolution profiles of paracetamol pellets.

By the application of canonical analysis, the significance of a global interdependence between the influencing variables, *X*, and the dependent variables, *Y*, are proved by the Wilks Λ test (a multivariate test of significance). Λ is approximated onto the *F*-distribution to reduce the number of dimensions and hence to ease interpretation, comparing the calculated value with the tabulated value for a first and second degree of freedom, f_1 and f_2, at a given level of significance (P; $P < 0.05$). The interpretation of the result of this multivariate analysis, as described by Podczeck et al. (1993), can be made by considering the following useful values: the extracting measures ($g^2_{X/V}$ and $g^2_{Y/V}$) indicating which part of the whole variance of one range (*X* or *Y*) can be explained by the canonical variables (*U* or *V*) of the same range; the "global" measures of redundancy ($g^2_{X/V}$ and $g^2_{Y/U}$) describing which part of the whole variance of one range can be explained by the canonical variables of the other range; and the interranging communalities ($d^2_{X/V}$ and $d^2_{Y/U}$) presenting the part of variance of one variable which can be explained by the canonical variables of the other range. The complete canonical solution is achieved if firstly $g^2_{X/U}$ is 1.0 and $g^2_{Y/V}$ is 1.0 or less which infers that the experimental design was well balanced, and secondly the "global" measures of redundancy, $g^2_{X/V}$ and $g^2_{Y/U}$ are less than 1.0. For further discussion, however, $g^2_{Y/U}$ will be considered while $g^2_{X/V}$ is not relevant as the variables studied were chosen to evaluate whether, and to what extent, formulation variables or independent variables influenced the dependent variables rather than to make an empirical prediction about the independent variables from the results of dependent variables. In all cases, the extracting measuring values $g^2_{X/U}$ are 1.0 and $g^2_{Y/V}$ are less than 1.0 (Table 8) indicating that the investigations were well designed and led to complete canonical solutions.

Considering the first section of the canonical analysis (Table 8), the Wilk's test Λ ($\Lambda = 0.00002$; approximate *F*-value = 53.06; $f_1 = 25$, $f_2 = 76$; $P < 0.001$) signifies a global interdependence between the independent variables (*X*) or formulation variables and the dependent variables (*Y*), including the values of BL, SSF, perimeter, density and porosity of pellets. The "global" measure of redundancy, $g^2_{Y/U}$ indicates that not more than 56.8% of the values of dependent variables can be explained by the canonical variables of *X* (*U*). Therefore, there must be some other uncontrolled factors, which influenced this group of dependent variables but which have not been identified. Comparing the interranging communalities, the result shows that each dependent variable was significantly influenced by some of the influencing factors. The influence on the value of BL ($d^2_{BL/U} = 0.841$) was greater than that on the density ($d^2_{density/U} = 0.730$), porosity ($d^2_{porosity/U} = 0.691$), the perimeter ($d^2_{perimeter/U} = 0.664$) of pellets and the value of SSF ($d^2_{SSF/U} = 0.595$). The interranging communalities further indicate that the main influencing factors of these dependent variables were the levels of paracetamol ($d^2_{LP/V} = 0.964$), chitosan ($d^2_{LP/V} = 0.835$) and sodium alginate ($d^2_{LSA/V} = 0.816$). The

Table 8
The results of canonical analysis applied to influencing factors, X (LP, LA, LC, LSA, PH) and dependent factors, Y (BL, SSF, perimeter, density, porosity)

Parameter	Canonical analysis	F-value	Significance level (P)
Test of significance			
Λ	0.00002	53.06*	<0.001
Extracting measures			
$g^2_{X/U}$	1.000		
$g^2_{Y/V}$	0.796		
"Global" measures of redundancy			
$g^2_{X/V}$	0.494		
$g^2_{Y/U}$	0.568		
Interranging communalities			
$d^2_{X/V}$			
$d^2_{\mathrm{LP}/V}$	0.964	64.05	<0.001
$d^2_{\mathrm{LA}/V}$	0.679	4.11	0.008
$d^2_{\mathrm{LC}/V}$	0.835	11.07	<0.001
$d^2_{\mathrm{LSA}/V}$	0.816	9.54	<0.001
$d^2_{\mathrm{PH}/V}$	0.345	1.00	n.s.
$d^2_{Y/V}$			
$d^2_{\mathrm{BL}/U}$	0.841	11.60	<0.001
$d^2_{\mathrm{SSF}/U}$	0.595	2.63	0.049
$d^2_{\mathrm{perimeter}/U}$	0.664	3.78	0.011
$d^2_{\mathrm{density}/U}$	0.730	5.47	0.002
$d^2_{\mathrm{porosity}/U}$	0.691	4.39	0.006

LP: level of paracetamol; LA: level of microcrystalline cellulose (Avicel PH101); LC: level of chitosan; LSA: level of sodium alginate; PH: pH level of binder liquid; BL, the "best" binder liquid level; SSF: steady-state extrusion force for the "best" formulation.
* Approximate F-value with f_1; f_2 (25; 76). n.s., not significant ($P > 0.05$).

level of MCC ($d^2_{\mathrm{LA}/V} = 0.679$) can also be related to the behaviour of these formulations, although it was a less important factor. The pH level of binder liquid ($d^2_{\mathrm{PH}/V} = 0.345$) did not have a significant influence on any of the responses.

Looking at the result of canonical analysis in the second section (Table 9), the Wilk's test ($\Lambda = 0.202$; approximate F-value =5.63; $f_1 = 10$, $f_2 = 46$; $P < 0.001$) indicates a global interdependence between the formulation variables and the dependent variables, including the values of d_{m} and shape factor of pellets. The value of 21.1% of the "global" measure of redundancy, $g^2_{Y/U}$ reflects that a good overall prediction of dependent variables by the canonical variables of X cannot be achieved. However, it can be seen from

Table 9
The results of canonical analysis applied to influencing factors, X (LP, LA, LC, LSA, PH) and dependent factors, Y (shape, size)

Parameter	Canonical analysis	F-value	Significance level (P)
Test of significance			
Λ	0.202	5.63*	<0.001
Extracting measures			
$g^2_{X/U}$	1.000		
$g^2_{Y/V}$	0.270		
"Global" measures of redundancy			
$g^2_{X/V}$	0.344		
$g^2_{Y/U}$	0.211		
Interranging communalities			
$d^2_{X/V}$			
$d^2_{\mathrm{LP}/V}$	0.056	1.00	n.s.
$d^2_{\mathrm{LA}/V}$	0.098	1.00	n.s.
$d^2_{\mathrm{LC}/V}$	0.612	8.08	0.002
$d^2_{\mathrm{LSA}/V}$	0.844	33.55	<0.001
$d^2_{\mathrm{PH}/V}$	0.100	1.00	n.s.
$d^2_{Y/U}$			
$d^2_{\mathrm{size}/U}$	0.796	8.33	<0.001
$d^2_{\mathrm{shape}/U}$	0.369	1.00	n.s.

LP: level of paracetamol; LA: level of microcrystalline cellulose (Avicel PH101); LC: level of chitosan; LSA: level of sodium alginate; PH: pH level of binder liquid; size: geometric mean pellet diameter; shape: shape factor.
* Approximate F-value with f_1; f_2 (10; 46). n.s., not significant ($P > 0.05$).

the interranging communalities that the shape factor of pellets ($d^2_{\mathrm{shape}/U} = 0.369$) was not significantly influenced by the formulation variables, while the median pellet size ($d^2_{\mathrm{size}/U} = 0.796$) was related to some of the formulation variables, namely the levels of sodium alginate ($d^2_{\mathrm{LSA}/V} = 0.844$) and chitosan ($d^2_{\mathrm{LC}/V} = 0.612$). The results for the shape factor are reasonable because the pellet formulations were optimized for the "best" shape, i.e. being round. Hence, any true relationship should be restricted by the choice of samples chosen.

In the last section of canonical analysis (Table 10), the relationship between the formulation variables and the characteristics of dissolution time profiles of paracetamol release was considered. The Wilk's test ($\Lambda = 0.104$; approximate F-value = 9.23; $f_1 = 10$, $f_2 = 44$; $P < 0.001$) signifies the interdependence between the formulation variables and the dependent variables including the values of AUC and MDT.

Table 10
The results of canonical analysis applied to influencing factors, X (LP, LA, LC, LSA, PH) and dependent factors, Y (AUC, MDT)

Parameter	Canonical analysis	F-value	Significance level (P)
Test of significance			
Λ	0.104	9.23*	<0.001
Extracting measures			
$g^2_{X/U}$	1.000		
$g^2_{Y/V}$	0.465		
"Global" measures of redundancy			
$g^2_{X/V}$	0.288		
$g^2_{Y/U}$	0.818		
Interranging communalities			
$d^2_{X/V}$			
$d^2_{\mathrm{LP}/V}$	0.753	17.04	<0.001
$d^2_{\mathrm{LA}/V}$	0.373	1.00	n.s.
$d^2_{\mathrm{LC}/V}$	0.660	10.04	0.001
$d^2_{\mathrm{LSA}/V}$	0.512	4.61	0.019
$d^2_{\mathrm{PH}/V}$	0.189	1.00	n.s.
$d^2_{Y/U}$			
$d^2_{\mathrm{AUC}/U}$	0.905	20.76	<0.001
$d^2_{\mathrm{MDT}/U}$	0.905	20.72	<0.001

LP: level of paracetamol; LA: level of microcrystalline cellulose (Avicel PH101); LC: level of chitosan; LSA: level of sodium alginate; PH: pH level of binder liquid; AUC: area under the dissolution time curve; MDT: mean dissolution time.

* Approximate F-value with f_1; f_2 (10; 44). n.s., not significant ($P > 0.05$).

The "global" measure of redundancy, $g^2_{Y/U}$ indicates that the predictability of 81.8% for the values of the AUC and MDT from the canonical variables of X can be achieved. Comparing the interranging communalities, it can be inferred that the values of AUC and MDT were equally influenced ($d^2_{\mathrm{AUC/U}} = 0.905$, $d^2_{\mathrm{MDT}/U} = 0.905$). Further, the result indicates that the main influencing factor of these parameters was the value of LP ($d^2_{\mathrm{LP}/V} = 0.753$). The significant influence of the level of chitosan ($d^2_{\mathrm{LC}/V} = 0.660$) and sodium alginate ($d^2_{\mathrm{LA}/V} = 0.512$) also existed but were less important. No significant influence can be related to the level of MCC ($d^2_{\mathrm{LA}/V} = 0.373$) and the pH of binder liquids ($d^2_{\mathrm{PH}/V} \simeq 0.189$).

3.2.3. Multiple regression analysis

As suggested by the result of the previous statistical analyses, only the relationships between the variables and binding liquid level, pellet size and dissolution, in terms of AUC and MDT have potential quantifiable relationships.

The "best" binder liquid level (BL) is significantly correlated to two formulation variables, the levels of MCC and chitosan ($F = 52.34$; $f_1 = 2$, $f_2 = 21$; $P < 0.001$). The adjusted coefficient of determination (B_{adjusted}) of 0.817 shows an acceptable correlation, although the calculated root mean square (RMS) of 16.02% indicates a certain lack of fit of the model. It can be seen from the multiple regression equation:

$$\mathrm{BL} = 0.376\mathrm{LA} + 13.505 \ln \mathrm{LC} + 18.464$$

that both the values of LC and LA are the main factors. The increase in the values of LC and/or LA in the formulation results in an increase in the value of BL required. Nevertheless, due to the logarithmic component of LC, the extent of increase in the value of BL is smaller at the relatively higher level of chitosan.

The median size of pellets is significantly correlated to the levels of added polymers ($F = 145.84$; $f_1 = 3$, $f_2 = 26$; $P < 0.001$). The multiple regression equation:

$$\mathrm{MEDIAN\ SIZE} = 70.131\mathrm{LSA} - 1.793\mathrm{LSA}^2 + 48.525\mathrm{LC} \times \mathrm{LSA} + 1341.2$$

has the B_{adjusted} value of 0.937 and RMS of 9.58% indicates a good correlation of the model. The value of LSA is shown to be an important factor affecting the median pellet size. With a negative quadratic term, the relationship between the value of LSA and the median pellet diameter is a parabolic shaped downward relationship. There is also a first-degree interaction between the values of LSA and LC with a positive regression coefficient. Therefore, the change in the median pellet size when varying the value of LSA is dependent on the value of LC in the formulation.

The characteristics of the dissolution profiles for the paracetamol release, the values of AUC ($F = 33.98$; $f_1 = 3$, $f_2 = 25$; $P < 0.001$) and MDT ($F = 33.42$; $f_1 = 3$, $f_2 = 25$; $P < 0.001$), are significantly correlated to the values of LP, LC and LSA. The B_{adjusted} values of 0.779 and 0.776 for the regression lines of the AUC value and the MDT value, respectively, reflect the relatively good correlation, although the RMS values of 47.45 and 47.31% for the correlation models of the AUC value and the MDT value, respectively,

show the lack of fit of both models. It can be seen from the multiple regression equation:

$$AUC = 1.493LP - 1.941LC + 0.852LSA + 11.348$$

$$MDT = 0.016LP - 0.020LC + 0.009LSA + 0.117$$

that the values of AUC and MDT are positively related to the values of LP and LSA and the effect of the LP value is slightly more distinct. The values of AUC and MDT also negatively respond to the change of the LC value in the formulation.

4. Conclusions

The statistically designed experiment was able to indicate the important formulation variables, which influenced the formation of pellets prepared by the process of extrusion/spheronisation and the in vitro paracetamol release. In these experiments extrusion was with a ram extruder fitted with a 1 mm die, which has a length to radius ratio of 8. It has been shown that it was possible to prepare pellets by varying the range of formulation variables: 2.5–40% of paracetamol, 0–16% of chitosan, 0–16% of sodium alginate, 30–70% of microcrystalline cellulose and the pH 2.2–5.4 of binder liquids, without an overall influence on the sphericity of pellets. Analysis of the results using the values determined in preparing the pellets with the highest value for the shape factor to identify the 'best' level of binder liquid, indicated that, formulation variables, except the pH of binder liquids, affected the binder liquid level required to form spherical pellets, the steady-state extrusion force and the perimeter, density and porosity of pellets. The levels of paracetamol, chitosan and sodium alginate were identified to be the main factors involved in the behaviour of these formulations, while the level of microcrystalline cellulose (Avicel PH101) was less important but still significant. The size of pellets could only be related to the addition of chitosan and sodium alginate into the formulation. The levels of paracetamol, chitosan and sodium alginate significantly influenced the in vitro dissolution of paracetamol pellets. The disintegration of pellets containing chitosan was observed. The release rate mechanism of each formulation was different and associated with the levels of formulation variables; however, the diffusion control generally occurred when pellets remained intact. There was no implication of the interaction between oppositely charged polymers, in terms of retarding the drug release from pellets.

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