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Effect of matrix composition and process conditions on casein–gelatin beads floating properties

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Abstract

Casein–gelatin beads have been prepared by emulsification extraction method and cross-linked with D,L-glyceraldehyde in an acetone–water mixture 3:1 (v/v). Casein emulsifying properties cause air bubble incorporation and the formation of large holes in the beads. The high porosity of the matrix influences the bead properties such as drug loading, drug release and floatation. These effects have been stressed by comparison with low porous beads, artificially prepared without cavities. The percentage of casein in the matrix increases the drug loading of both low and high porous matrices, although the loading of high porous matrices is lower than that of low porous matrices. As a matter of fact, the drug should be more easily removed during washing and recovery because of the higher superficial pore area of the beads. This can explain the drug release rate increase, observed in high porous matrix, in comparison with beads without cavities. This is due to the rapid diffusion of the drug through water filled pores. The study shows that cavities act as an air reservoir and enable beads to float. Therefore, casein seems to be a material suitable to the inexpensive formation of an air reservoir for floating systems.

Keywords: Oral drug delivery systems; Casein; Gelatin; Floating beads

1. Introduction

Controlled release systems attempt primarily to control drug concentration in the target tissue reducing the number of administrations, to ensure patient compliance and to improve efficacy of drugs (Chien, 1992).

Over the past 20 years several controlled delivery systems have been formulated. A branch of research has been dedicated to those able to be retained in the stomach for a long time. Among the different dosage forms for prolonged gastric residence, our study has been dedicated to floating systems. Such systems seem to be useful for drugs acting locally in the proximal gastrointestinal tract and drugs unstable in intestinal fluids, but well absorbable in the stomach.

Many sophisticated techniques have been applied to prepare experimental floating devices, generally consisting of a capsule containing a drug

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reservoir and a floating reservoir filled with gas (Buri, 1985) or containing a carbon dioxide generating blend (Ingani et al., 1987). A simple method for producing floating systems uses polymeric materials, having a density lower than that of the gastric fluid (Buri, 1985).

In spite of all these sophisticated formulations, the retention time of these systems depends on many physiologic factors. It is well known that an early gastric emptying of a monolithic device causes a rapid lack of efficacy, in case of a drug having only an absorption window in the stomach.

Multi-particulate floating systems have been proposed to undergo this problem. Multi-particulate floating systems, in fact, distribute uniformly into the gastric content and gradually empty from the stomach, resulting in a long lasting effect (Daumesnil, 1994).

Polymeric microspheres have attracted considerable attention as drug carriers for controlled release systems (Davis et al. 1984). Particulate carriers are ideal in providing a constant therapeutic and non-toxic level of the drug. Various protein, polysaccharide and other polymeric materials have been investigated as drug carriers, and many techniques have been pointed out to synthesise nanoparticles, microparticles and beads (Doelker, 1985).

Casein has recently attracted increasing attention to prepare biodegradable microspheres by emulsion technique (Chen et al., 1987; Latha and Jayakrishnan, 1994). In those works casein solutions are usually prepared in NaOH, where casein is very soluble. According to these Authors, the microspheres so obtained do not show any floating properties.

In the food industry, casein is widely used as a functional surface active protein. Proteins in fact are well known to provide various textures and physical stability to food systems.

The amphipathic nature of proteins, arising from the mixture of polar and non-polar aminoacid residues, causes them to concentrate at air–water or oil–water interfaces and to reduce surface or interfacial tension and hence to reduce the mechanical energy required to form a foam or emulsion. The foaming properties of proteins are usually characterised in terms of foam volume, over-run, foam strength, drainage rate, breakdown time and half-life period of the foam.

Casein is often used as soluble sodium caseinate (Na-caseinate) (Southward and Walker, 1980), therefore, the emulsifying and foaming properties of Na-caseinate have been widely studied. To our knowledge, in the preparation of floating beads containing air reservoirs, no studies on the application of casein foaming properties have been reported.

This paper aims to evaluate the influence of casein concentration on air emulsion, floating properties, loading and drug release. For this purpose, casein foaming properties have been studied in terms of foam drainage rate, whereas mixtures at different ratios of soluble casein and gelatin are used to recover two kinds of systems: beads prepared at room pressure and beads obtained under vacuum.

2. Materials and methods

2.1. Materials

Soluble casein (light white; batch 9211260D, BDH Chemicals, Poole, England) and gelatin (225 Bloom) from calf skin (batch CW 05314MV, Aldrich Chemicals, Milwaukee, WI), D,L-glyceraldehyde (2,3-dihydroxypropionaldehyde, MW 90.08, Aldrich Chemicals), mineral oil (Carlo Erba, Milan, Italy) were used as received from the manufacturers. Fluorescein sodium salt (FluNa) (MW 376.3, RS, Carlo Erba) was used as model drug. Simulated gastric fluid at pH 1.2 (without pepsine) was prepared as reported in US Pharmacopoeia.

2.2. Material characterisation: casein foaming properties

The casein emulsifying properties have been studied in terms of serum drainage of casein-stabilised foams.

Casein solutions were prepared in deionized water. Four casein concentrations were chosen corresponding to the casein percentage in bead

preparative solution (3; 7.5; 10; 15%, w/v) and a lower percentage (1%, w/v). The solutions were stored for 16 h at 4°C and brought to room temperature at least 1 h before usage (Britten and Lavoie, 1992).

Foam was developed according to the method proposed from Mohanty et al. (1988). A 50 ml sample was whipped at 20 ± 1°C in a blender (Ika-Werk, Stanfen, Germany) at 2400 rev/min in a 150 ml beaker for 5 min and, immediately, transferred to a 150 ml graduated cylinder and the total volume of foam and liquid noted. Kinetics values of serum drainage were calculated from the change in total volume and serum volume at prefixed intervals (Elizalde et al., 1991).

All the data averaged at least three determinations.

2.3. Preparation of beads

2.3.1. Preparation of uncross-linked beads

The preparation process is based on the method described by Tanaka et al. (1963).

A 30% w/v solution in deionized water (20 ml) at 60°C containing mixtures of casein and gelatin (ratios in Table 1) was added dropwise to mineral oil (60 ml) preheated to 60°C.

The dispersion was stirred using a paddle stirrer, holding constant dispersion time (10 min) and speed at 500 rev/min.

As the emulsion was obtained, the temperature was lowered to 5°C by rapid cooling in an ice bath. Then, to solidify the droplets in the dispersed phase completely, 100 ml of previously cooled (5°C) acetone was added and the stirring was continued for 15 min. The solidified beads were recovered by filtration and vacuum dried (10 mm Hg) at room temperature. After drying the beads were sieved in order to obtain the fraction with diameter between 1 and 2 mm.

The batches were called Cagel.

Table 1
Batch name according to the casein–gelatin ratio in the preparative mixture (the corresponding casein percentage in parentheses)

Batch name		casein–gelatin ratio
Cagel (1:9)	Degas (1:9)	1:9 (casein 10% p/p)
Cagel (1:3)	Degas (1:3)	1:3 (casein 25% p/p)
Cagel (1:2)	Degas (1:2)	1:2 (casein 33% p/p)
Cagel (1:1)	Degas (1:1)	1:1 (casein 50% p/p)

2.3.2. Vacuum preparation of uncross-linked beads

The aqueous solutions, containing mixtures of casein and gelatin (ratios in Table 1), were treated at reduced pressure (10 mmHg) up to complete removal of air bubbles. The vacuum treated solutions were used for the preparation of the beads, as reported above for the 'Section 2.3.1'.

These batches were called Degas.

2.3.3. Cross-linking of beads

The beads (Cagel and Degas) (1 g) were cross-linked (6 h) by suspending them under magnetic stirring (400 rev/min) in mixture acetone–water 3:1 (v/v) containing glyceraldehyde (0.5%, w/v). The bead samples were filtered, washed with 20 ml of cool acetone (5°C) and vacuum dried (10 mm Hg) at room temperature.

2.3.4. Incorporation of Sodium Fluorescein

The cross-linked beads (Cagel and Degas) (1 g) were suspended in FluNa aqueous solution (40 ml) at a concentration of 2% (w/v) over the same time (1 h) at room temperature in continuos stirring (300 rpm). After the addition of acetone (100 ml), beads were filtered and washed with acetone twice. Beads were vacuum-dried at room temperature.

2.4. Characterisation of beads

2.4.1. Morphological aspect

The morphological aspect was determined by scanning electron microscopy (Model XL40, Philips, Eindohven, The Netherlands).

The beads were put onto a double sided tape on an aluminum stub, sputter coated with gold–palladium in an argon atmosphere and examined at 15 kV.

2.4.2. Porosity

The porosity was determined by mercury intrusion (Autopore 9215 II, Micromeritics, Atlanta,

GA). This technique is based on the fact that the P pressure required to drive mercury through a pore increases as the pore diameter decreases, as described by the Washburn equation (Miller et al., 1983):

$$P = (-4\sigma \cos\theta)/d \quad (1)$$

where d is the pore diameter, σ is the mercury/air interfacial tension and θ is the contact angle at the mercury/air/pores wall interface. A plot of the volume of mercury versus pressure is a common way to display the raw data. The shape of the porosimetry curve provides information about the pore morphology.

2.4.3. Density evaluation

The density values of the raw materials (soluble casein and gelatin), the ones of their physical mixtures at the same casein–gelatin ratios used for the beads preparation and the ones of the cross-linked unloaded beads (Cagel and Degas) were measured using an air comparison pycnometer (model 930, Beckman, Fullerton, CA). All the values were the average of at least ten determinations.

2.4.4. Measurement of the bead buoyancy in simulated gastric fluid

The buoyancy of the beads (Cagel and Degas) was studied at 37 ± 0.2°C, soaking 100 beads in 100 ml of simulated gastric fluid at pH 1.2 (without pepsine) (US Pharmacopoeia). The number of floating beads on the buffer surface was evaluated at fixed time intervals. The floating time (Tfloat.) was considered as the time at which the 100% of the beads floated. All the data are the average of at least three determinations.

2.5. Analysis of the drug content

The amount of FluNa incorporated was determined digesting beads (Cagel and Degas) (100 mg) in NaOH 1N (100 ml). The suspension was incubated at room temperature until the beads were digested (48 h). The solution was filtered through a 0.45 μm filter membrane and diluted 1:100 (v/v) in deionized water and spectrophotometrically analysed at 491 nm.

Controls were performed with pure drug and unloaded beads incubated separately in 1 N NaOH. Neither FluNa degradation nor protein interferences were observed.

2.6. Drug release

Approximately 100 mg of the FluNa loaded beads (Cagel and Degas) were suspended in 900 ml simulated gastric fluid at pH 1.2 (US Pharmacopoeia) in Apparatus II (US Pharmacopoeia) at 37 ± 0.2°C under sink conditions. Aliquots of 3 ml were withdrawn at various time intervals, filtered and analysed spectrophotometrically at 492 nm and then reintroduced into the medium to maintain constant volume.

2.7. Release kinetics analysis

Release data were analysed according to the Langenbucher quantitative interpretation (1972):

$$\log[-\ln(1-m)] = \log(t - T_i) - \log(T_d)b$$

where m is the related fraction at time t; b is the shape parameter characterising the release curve; T_i is the location parameter representing the time lag before the actual onset of the release process which, in most cases, will be equal to zero; T_d is the dissolution time, i.e. the time interval necessary to dissolve 63.2% of the drug.

3. Results and discussion

3.1. Bead characterisation

The morphological analysis of Cagel beads showed a nearly spherical shape and a rough surface without any pore (Fig. 1(a)). The sections of cross-linked and uncross-linked beads show the presence of spherical cavity with a random distribution. The hole-number appears dependent on the casein concentration in the matrix, as presented in Fig. 1(b and c).

It can be reasonable to hypothesise that air bubbles introduced in the solution during the preparation process are emulsified by casein, up to the complete gelation of the protein solution after cooling.

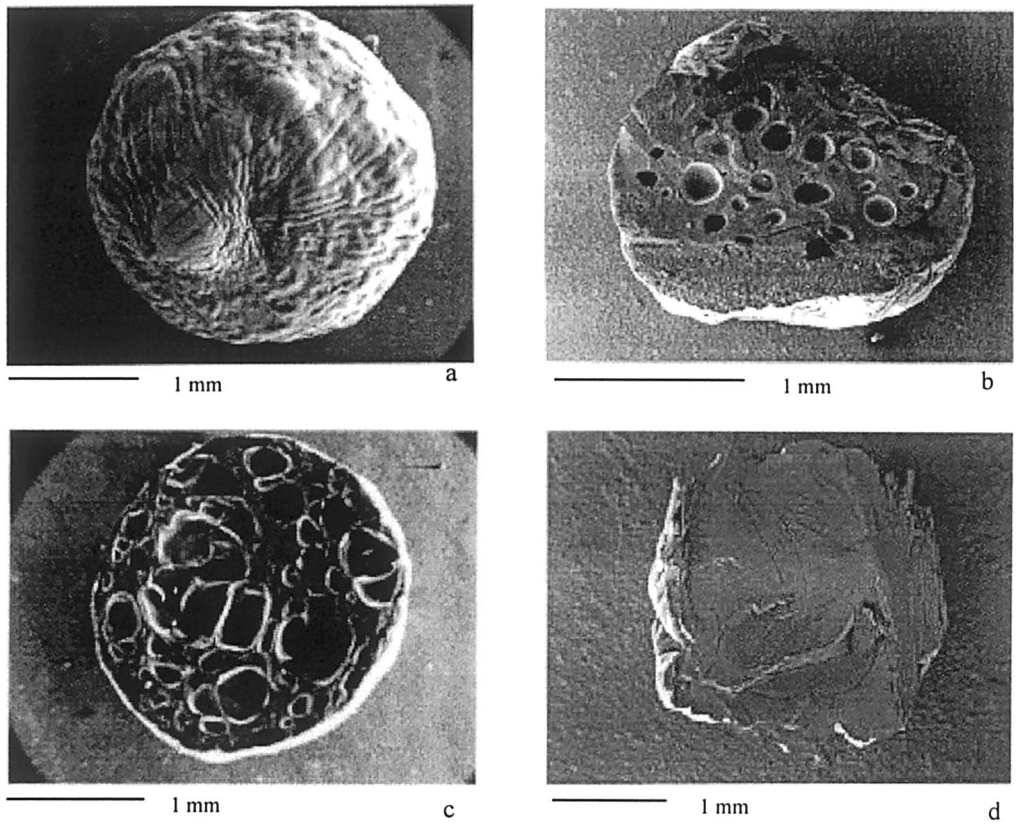

Fig. 1. (a) Surface of Cagel bead. (b) Section of Cagel (1:9). (c) Section of Cagel (1:1). (d) Section of Degas bead.

In order to study the stabilising effect of proteins on serum drainage, several empirical equations have been reported (Mita et al., 1977; Kim and Kinsella, 1985). Elizalde et al. (1991) recently proposed the following equation to express the rate of serum drainage on casein-stabilised foams.

$$v(t) = V_t/(B + t)$$

where $v(t)$ is the rate of drained serum at time t; V_t is the maximum volume of drained serum; and B is the time needed to drain $V/2$. The initial rate of drainage (R_0) was estimated from the equation $R_0 = V/B$, derived from the equation previously reported (Elizalde et al., 1991). R_0 has been chosen as a parameter to evaluate stabilising properties of casein on emulsion. Initial drainage rates were plotted as a function of protein concentration (Fig. 2). The increase in protein concentration resulted in a decreased rate of drainage and allowed more water to remain in the foam matrix. This effect can explain the casein dependent air

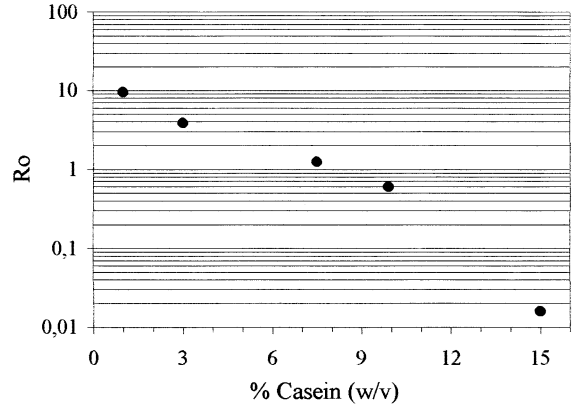

Fig. 2. Initial rate of serum drainage (R_0) of a foam stabilised by casein as a function of casein percentage.

Table 2
Porosity percentage of the uncross-linked and cross-linked beads

Batch	Uncross-linked beads	Cross-linked beads
Cagel (1:9)	33	35
Cagel (1:3)	48	53
Cagel (1:2)	55	58
Cagel (1:1)	58	51

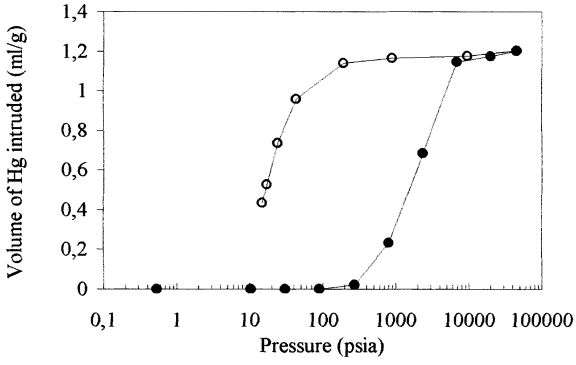

Fig. 3. Intrusion–extrusion curve of cross-linked Cagel beads. Lines are drawn for clarity, but do not indicate curve fitting. Key: (●) intrusion, (○) extrusion.

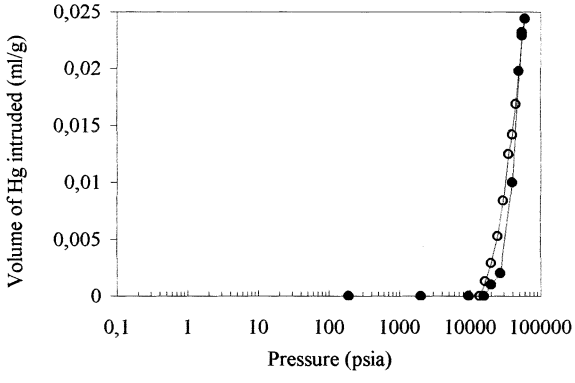

Fig. 4. Intrusion–extrusion curve of cross-linked Degas beads. Lines are drawn for clarity, but do not indicate curve fitting. Key: (●) intrusion, (○) extrusion.

bubbles incorporation in Cagel. For a better understanding of this behaviour, Degas beads have been prepared from vacuum treated casein–gelatin solutions.

The vacuum treatment eliminates air bubbles incorporated during the preparation of the protein solutions; in fact microphotographs of Degas sections show the absence of cavities (Fig. 1(d)).

The emulsifying effect of casein is made apparent by comparison between porosity data of Cagel and Degas. Namely, the Cagel porosity data show that matrix porosity increases according to the casein percentage (Table 2), on the contrary Degas have a 2% porosity independent on the matrix composition.

The shape of the porosimetry curves (volume of mercury vs. pressure) provides information about pore conformation. Figs. 3 and 4 represent the intrusion–extrusion curves, respectively of Cagel and Degas samples.

The diagram of Degas represents a sample containing essentially a narrow size range of regular pores, as indicated by only one rapid increase in volume intruded and the complete mercury extrusion (Fig. 4). The observed hysteresis in Cagel diagram (Fig. 3), caused by mercury being permanently trapped in the pores of the sample, occurs where constrictions and random topology occur. Consequently, the Cagel extrusion curves indicate the presence of pores in the matrix connected to each other and the surface by throats. We can suppose that the bigger pores derive from air bubbles, random incorporated during the preparation of the protein solution, and throats may correspond to the cylindrical pores, being in under vacuum treated beads.

The comparison between Cagel porosity data (Table 2) and density data (Table 3) stresses the importance of air incorporation. The density of the physical mixtures decreases as the casein percentage increases, because the density of casein being lower than that of gelatin. The density of uncross-linked and cross-linked beads is markedly lower than that of the corresponding physical mixture. This observation can be explained by the air bubbles incorporation, enhanced by casein. Moreover, comparing cross-linked and uncross-linked beads a small density increase is observed, but no porosity reduction.

The comparison between Cagel and Degas density data (Tables 3 and 4, respectively) shows how air incorporation is the factor affecting the density

Table 3
Density (g/cm^3) of the physical mixture, the ones of Cagel beads uncross-linked and cross-linked (standard deviations in parentheses)

Bach	Physical mixture	Uncross-linked beads	Cross-linked beads
Gelatin	1.42 (±0.02)	–	–
Cagel (1:9)	1.33 (±0.02)	0.85 (±0.04)	1.04 (±0.03)
Cagel (1:3)	1.20 (±0.05)	0.79 (±0.03)	0.99 (±0.03)
Cagel (1:2)	1.12 (±0.03)	0.74 (±0.02)	0.86 (±0.03)
Cagel (1:1)	1.05 (±0.04)	0.66 (±0.08)	0.75 (±0.03)
Casein	0.83 (±0.11)	–	–

differences of batches in a stronger fashion than the variation between the density values of casein and gelatin. In fact, no density differences are observed among Degas batches as casein percentage increases.

Degas batches do not float, as we can assume comparing density values, whereas the density values justify the observed buoyancy of Cagel batches (Fig. 5). The Cagel floatation lag time is due to the time necessary to obtain the density decrease by matrix swelling in simulated gastric fluid. In fact, the floating process depends on the balance between the weight and the volume variations of the dosage forms (Timmermans and Moës, 1990a). The volume increase causes the resultant-weight increase and then the dosage form floatation, which has already been demonstrated by Timmermans and Moës (1990b).

3.2. Drug content and drug release

The influence of the porosity on drug content and drug release has been observed. The post-preparation loading is convenient, because drug is not exposed directly to the glyceraldehyde cross-linker, although the matrix composition could modify the drug penetration through the matrix, influencing the FluNa content. As a matter of fact, increasing casein percentage in Cagel beads, the FluNa content also increases (Table 5). According to the experimental data, almost all Cagel batches [except Cagel (1:2)] have a lower loading than Degas batches. This finding is probably caused by the easy removal of drug during washing and recovery, due to the higher superficial area per unit of mass originated by pores.

The drug release profiles of Cagel and Degas are markedly different (Figs. 6 and 7). The dissolution time (T_d) values of Cagel and Degas samples having the same composition (compared in Table 6) show how the drug release gets faster through a porous matrix.

Table 4
Density (g/cm^3) of uncross-linked and cross-linked Degas beads (standard deviations in parentheses)

Bach	Uncross-linked beads	Cross-linked beads
Degas (1:9)	1.36 (±0.01)	1.36 (±0.01)
Degas (1:3)	1.34 (±0.04)	1.43 (±0.03)
Degas (1:2)	1.33 (±0.01)	1.39 (±0.01)
Degas (1:1)	1.26 (±0.09)	1.36 (±0.02)

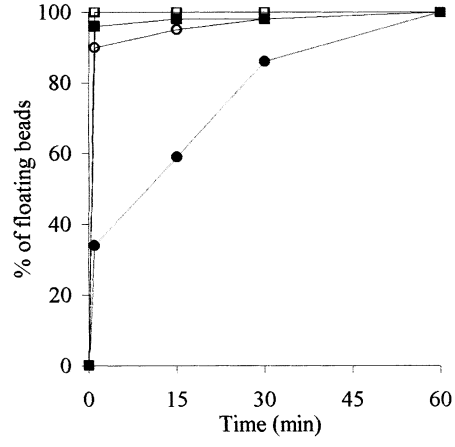

Fig. 5. Floating behaviour of cross-linked Cagel beads. All points represent the average of at least three measurements. Lines are drawn for clarity, but do not indicate curve fitting. Key: [●] Cagel (1:9); [○] Cagel (1:3); [■] Cagel (1:2); [□] Cagel (1:1).

Table 5
Comparison between FluNa content (mg/100mg) of Cagel and Degas beads[a]

Bach (casein–gelatin ratio)	FluNa content (mg/100mg)	
	Cagel	Degas
(1:9)	7.2 (±0.1)	9.2 (±0.1)
(1:3)	8.4 (±0.3)	14.3 (±0.1)
(1:2)	9.2 (±0.2)	10.3 (±1.0)
(1:1)	10.9 (±0.5)	14.3 (±0.2)

[a] Batches are compared as casein–gelatin ratios; standard deviations in parentheses.

This observation can be expected because in highly porous beads, the drug diffuses mainly through water filled pores. Moreover, in Cagel and Degas beads, it is possible to hypothesise that the drug is not only absorbed onto the pore surface, but a part of it is also dissolved in the matrix. In fact, the release rate of Cagel beads decreases as porosity increases, according to the increase of the casein percentage in the matrix composition. In fact increasing casein percentage in the matrix, the dissolution time (T_d) decreases both in Cagel and Degas samples. This result is probably due to the casein effect on cross-linking degree, as previously demonstrated (Bulgarelli et al., 1999). Moreover, these preliminary studies about porosity effects on drug release rate are now subject to further investigations.

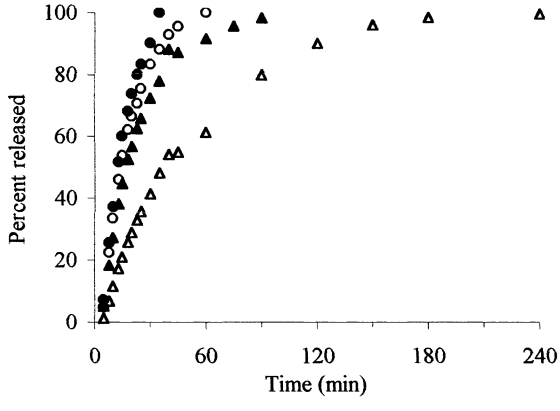

Fig. 6. Release profile of Cagel beads in simulated gastric fluid at pH 1.2 (without pepsine). Key: [●] Cagel (1:9); [○] Cagel (1:3); [▲] Cagel (1:2); [△] Cagel (1:1). All points represent the average of at least three determinations.

4. Conclusion

Casein is a simple and inexpensive material used in the preparation of controlled oral drug delivery systems, featuring flexibility in a variety of uses.

Casein emulsifying properties, in fact, cause air bubbles incorporation, that act as air reservoirs in floating systems. On the contrary, a simple variation in the preparation method, such as the vacuum treatment, bring about unfloating systems.

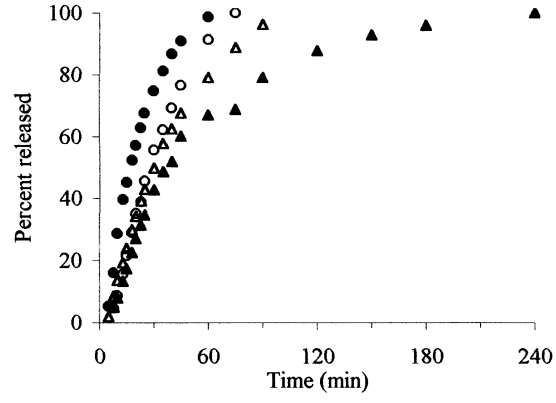

Fig. 7. Release profile of Degas beads in simulated gastric fluid at pH 1.2 (without pepsine). Key: [●] Degas (1:9); [○] Degas (1:3); [▲] Degas (1:2); [△] Degas (1:1). All points represent the average of at least three determinations.

Table 6
Parameters of the release process (T_d, min) calculated according to the Weibull function[a]

Bach (casein–gelatin ratio)	T_d (min)	
	Cagel	Degas
(1:9)	12 (±4)	20 (±3)
(1:3)	16 (±4)	30 (±2)
(1:2)	25 (±9)	53 (±6)
(1:1)	52 (±2)	37 (±4)

[a] Batches are compared as casein–gelatin ratios; 95% confidence limits in parentheses.

Both floating and unfloating systems are suitable to control drug release and are biodegradable, therefore they can be proposed as carrier for the oral administration of active agents.

Long lasting in the stomach floating systems or unfloating systems can be prepared alternately in order to deliver the drug, according to the therapy requirements.

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