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Preparation and in vitro evaluation of Eudragit microspheres containing acetazolamide☆

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Abstract

The aim of this study was to prepare and evaluate Eudragit (RS and RL) microspheres containing acetazolamide. Microspheres were prepared by solvent evaporation method using acetone/liquid paraffin system. The influence of formulation factors (stirring speed, polymer:drug ratio, type of polymer, ratio of the combination of polymers) on particle size, encapsulation efficiency and in vitro release characteristics of the microspheres were investigated. The yields of preparation and the encapsulation efficiencies were high for all formulations the microspheres were obtained. Mean particle size changed by changing the polymer:drug ratio or the stirring speed of the system. Although acetazolamide release rates from Eudragit RS microspheres were very slow and incomplete for all formulations, they were fast from Eudragit RL microspheres. When Eudragit RS was added to Eudragit RL microsphere formulations, release rates slowed down and achieved the release profile suitable for peroral administration.

Keywords: Acetazolamide; Eudragit; Microspheres; Controlled release

1. Introduction

Acetazolamide is an inhibitor of carbonic anhydrase and is used mainly in the management of glaucoma. It is also used in the treatment of various forms of epilepsy and to prevent or ameliorate the symptoms of acute high altitude sickness. However, it has dose related side effects, the most common of which are diuresis, gastric difficulties and metabolic acidosis (Martindale, 2001). Several topically applied formulations were developed in order to minimize its side effects. These include surfactant-gel preparations, contact lenses, aqueous solutions containing cyclodextrins and liposomes (El-Gazayerly and Hikal, 1997).

Garner et al. (1963) reported that the incidence of side effects was much lower and the tolerance was much greater with acetazolamide sustained release capsule when compared with the conventional acetazolamide tablet.

Microspheres are one of the multiparticulate delivery systems and are prepared to obtain prolonged or controlled drug delivery, to improve bioavailability or stability and to target drug to specific sites. Microspheres can also offer advantages like limiting fluctuation within therapeutic range, reducing side effects, decreasing dosing frequency and improving patient compliance (Davis and Illum, 1988; Ritschel, 1989).

Eudragit polymers are series of acrylate and methacrylate polymers available in different ionic forms. Eudragit RL and Eudragit RS are insoluble in aqueous media but they are permeable and both

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have pH-independent release profiles. The permeability of Eudragit RS and RL in aqueous media is due to the presence of quaternary ammonium groups in their structure; Eudragit RL has a greater proportion of these groups and as such is more permeable than Eudragit RS (Eudragit RS and RL data sheets).

The aim of this study was to prepare Eudragit microspheres containing acetazolamide to achieve a controlled drug release profile suitable for peroral administration. Firstly, we investigated some formulation variables (polymer type, polymer:drug ratio, stirring speed) to obtain spherical particles. Then yield of production, particle size distribution, encapsulation efficiency, surface properties and acetazolamide release rate from microspheres were investigated. The influences of formulation variables on the microsphere properties were examined and the microsphere formulations suitable to achieve our goal were determined.

2. Materials and methods

Eudragit RS and Eudragit RL, Röhm Pharma; acetazolamide, Sigma; magnesium stearate, Merck; *n*-hexane, Atabay; liquid paraffin, Laborlar. Other substances used were all of pharmaceutical grade.

2.1. Solubility measurement

Acetazolamide in an amount of excess of its solubility was added to 15 ml of dissolution medium in a 20 ml bottle maintained at $37 \pm 0.5\,^{\circ}C$ and shaken in a constant temperature water bath (Certomat WRB, Braun, Biotech International) for 48 h. At appropriate times aliquots of 1 ml were taken from the dissolution medium, filtered and then diluted to 100 ml. Drug content of the samples were assayed spectrophotometrically (Shimadzu UV 2100 S, Japan) at 265 nm. Calibration curves were used for the determination of the amounts dissolved.

2.2. Preparation of microspheres

Acetazolamide microspheres were prepared by solvent evaporation technique (Pongpaibul et al., 1988; Tsankov et al., 1992).

Different amounts of polymer (1, 2 or 3 g of Eudragit RS or Eudragit RL or a mixture of both) was dissolved in 27 ml of acetone by using a magnetic stirrer (Ikamag RH, Germany). Powdered acetazolamide (1 g) and magnesium stearate (100 mg) were dispersed in the polymer solution. The resulting dispersion was then poured into a vessel of 1000 ml containing the mixture of 270 ml liquid paraffin and 30 ml *n*-hexane while stirring. A cylindrical vessel (10 cm inside diameter and 14 cm height) and a mechanical stirrer with a blade (6 cm diameter) (RW 20 DZM, IKA, Germany) were used. Stirring was continued for an hour, until acetone evaporated completely. Polymer:drug ratio (1:1, 2:1 or 3:1, w/w) and stirring rate (500 and 750 rpm) of the system were changed to obtain spherical particles. After evaporation of acetone, the microspheres formed were collected by filtration in vacuum, washed 4–5 times with 50 ml *n*-hexane each and dried at room temperature for 24 h.

All microsphere formulations were prepared in triplicate.

Microspheres dried at room temperature were then weighed and the yield of microsphere preparation was calculated using the formula:

$$\text{Percent yield} = \frac{\text{the amount of microspheres obtained (g)}}{\text{the theoretical amount (g)}} \times 100.$$

2.3. Scanning electron microscope analysis

Shapes and surface characteristics of the microspheres were investigated and photographed using scanning electron microscope (SEM; Jeol JSM-6400, Japan).

2.4. Determination of the mean particle size

The microspheres were separated into different size fractions by sieving for 15 min using standard sieves having apertures of 425, 355, 250 and 180 μm (Retsch, Germany). The particle size distribution of the microspheres for all formulations determined and mean particle size of microspheres were calculated.

2.5. Encapsulation efficiency of the microspheres

Microspheres were crushed and powdered by using a mortar. Accurately weighed 100 mg of this powder was extracted in 100 ml of water. The solution was then filtered, a sample of 2 ml was withdrawn from

this solution, diluted to 50 ml with water and assayed spectrophotometrically at 265 nm to determine the acetazolamide content of the microspheres.

2.6. In vitro release studies

The dissolution rate of pure drug and the drug release rate from the microspheres were studied at pH 1.2 and 7.4 using the paddle method (US Pharmacopoeia XXIII, 1995) under sink conditions. Accurately weighed samples of acetazolamide or microspheres (size fraction 250 μm) were added to dissolution medium kept at 37 ± 0.5 °C. At preset time intervals aliquots were withdrawn and replaced by an equal volume of dissolution medium to maintain constant volume. After suitable dilution, the samples were analysed spectrophotometrically at 265 nm. The kinetics data obtained from release rates were also evaluated.

2.7. Statistical analysis

The data obtained from the particle size, encapsulation efficiency and release rate determination studies of acetazolamide microspheres were analysed statistically with one-way ANOVA and *t*-test by using SPSS for Windows 9.0.

3. Results and discussion

Solvent evaporation method was used to prepare acetazolamide microspheres. First, trials were made to prepare microspheres by using a solvent evaporation technique in the water phase, using acetone/water, alcohol/water or methylene chloride/water system; but although many formulations were investigated, no spherical particles could be obtained. Then acetone/liquid paraffin system was used and various formulations with different polymer:drug ratios were tried, stirring speed was also changed to obtain spherical particles. These microsphere formulations are shown in Table 1. When polymer:drug ratio was too low (1:1, w/w) no spherical particles were obtained independent of stirring speed of the system (750 or 500 rpm) and the type of polymer used (Eudragit RL or Eudragit RS). These results show that the amount of solid, thus the viscosity of the inner phase is an important factor for the preparation of microspheres. Keeping the drug amount and the solvent volume constant, spherical particles were obtained as the amount of polymer was increased to give a polymer:drug ratio of 2:1 (stirring speed 750 or 500 rpm) or 3:1 (stirring speed 750 rpm). However, when polymer:drug ratio was (3:1), the shapes of particles were irregular at 500 rpm, because for this high polymer concentration, this stirring speed was not fast enough to dispers the inner phase in the outer phase. Similar results were reported by Pongpaibul et al. (1988). When the stirring speed was raised to 750 rpm the best spherical particles with good surface characteristics were obtained with this polymer:drug ratio of 3:1. Two examples of the scanning electron micrographs of the microspheres prepared using two different formulations are shown in Figs. 1 and 2.

Table 1
Formulations of the microspheres prepared

No.	Eudragit RS (g)	Eudragit RL (g)	Magnesium stearate (mg)	Drug (g)	Stirring rate (rpm)
–[a]	1	–	100	1	750
–[a]	1	–	100	1	500
–[a]	–	1	100	1	750
–[a]	–	1	100	1	500
1	2	–	100	1	750
2	2	–	100	1	500
3	3	–	100	1	750
–[a]	3	–	100	1	500
4	–	2	100	1	750
5	–	2	100	1	500
6	–	3	100	1	750
–[a]	–	3	100	1	500
7	1.8	0.2	100	1	500
8	1.6	0.4	100	1	500
9	1.4	0.6	100	1	500
10	2.7	0.3	100	1	750
11	2.4	0.6	100	1	750
12	2.1	0.9	100	1	750

[a] No spherical microspheres were formed with these formulations.

Magnesium stearate was added to the formulations as droplet stabilizer to overcome the problem of droplet coalescence during solvent evaporation (Arshady, 1991).

Most of the microspheres were collected above the sieve of 250 μm by all formulations. The particle size distribution and mean particle size of the microspheres are shown in Figs. 3 and 4, respectively.

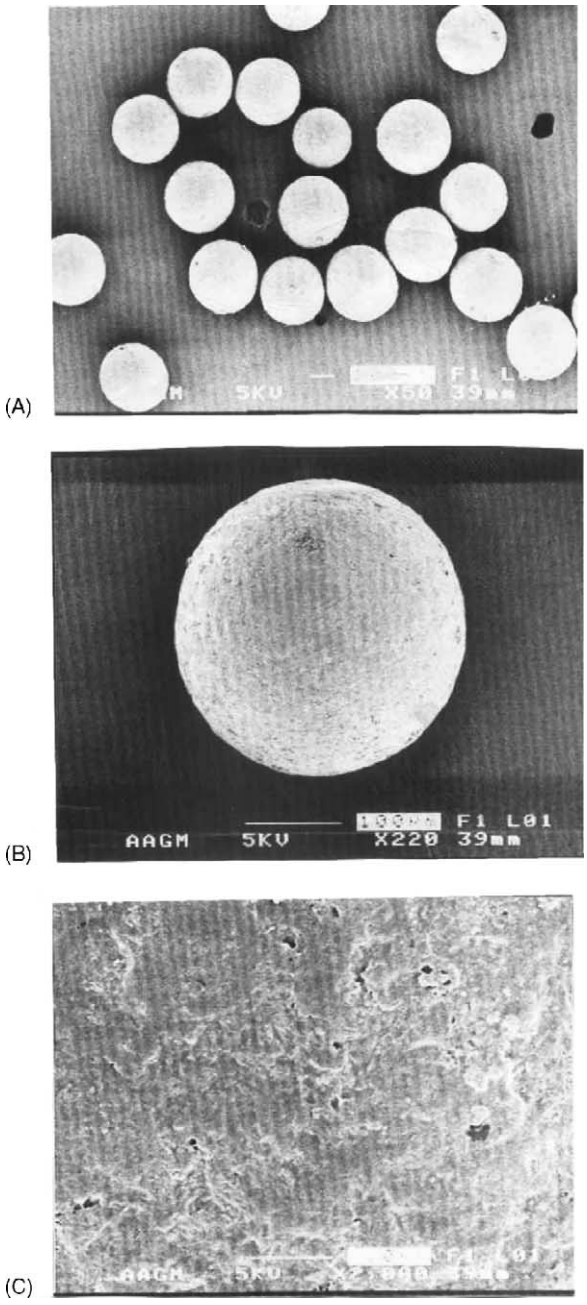

Fig. 1. Scanning electron micrographs of shape (A–B) and surface (C) of the microspheres (Eudragit RL:acetazolamide, 3:1; stirring speed: 750 rpm).

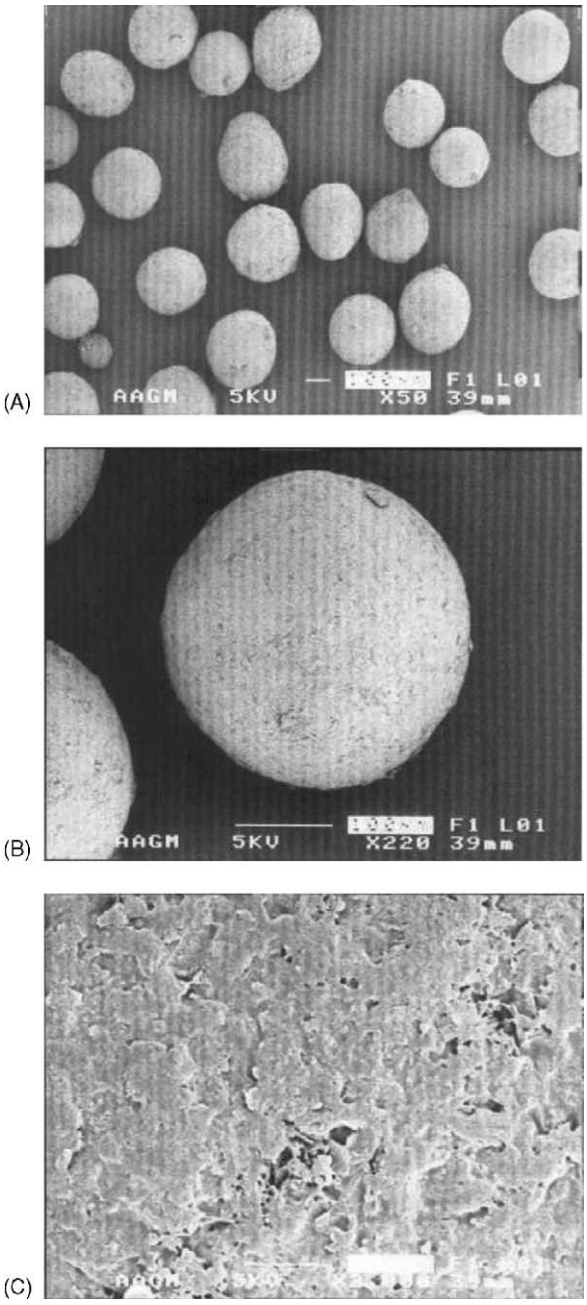

Fig. 2. Scanning electron micrographs of shape (A–B) and surface (C) of the microspheres (Eudragit RL:acetazolamide, 2:1; stirring speed: 500 rpm).

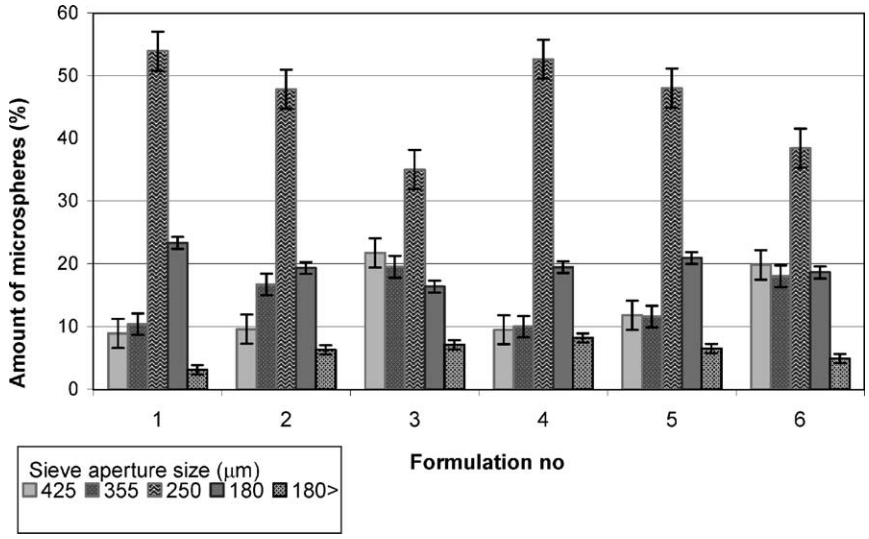

Fig. 3. Particle size distributions of the microspheres ($n = 3$).

According to Arshady (1990), various manufacturing parameters (apparatus design, type of stirrer, stirring speed, viscosity of emulsion phases and the stabilizer concentration) affect particle size. We have only investigated the effects of polymer concentration, thus the inner phase viscosity and the stirring speed of the system on particle formation and particle size, while keeping the other parameters constant. Increasing the polymer:drug ratio caused the mean microsphere size to shift towards a higher particle size ($P < 0.05$) as shown in Fig. 4. Higher concentration of polymer produced a more viscous dispersion which formed larger droplets and consequently larger microspheres as reported by Pongpaibul et al. (1984).

Increasing the speed of stirring decreased the particle sizes of microspheres ($P < 0.01$) as also reported by Babay et al. (1988).

The mean particle sizes were not affected by the polymer type ($P > 0.05$) and the ratio of the mixture of polymers ($P > 0.05$) for all formulations (Fig. 4).

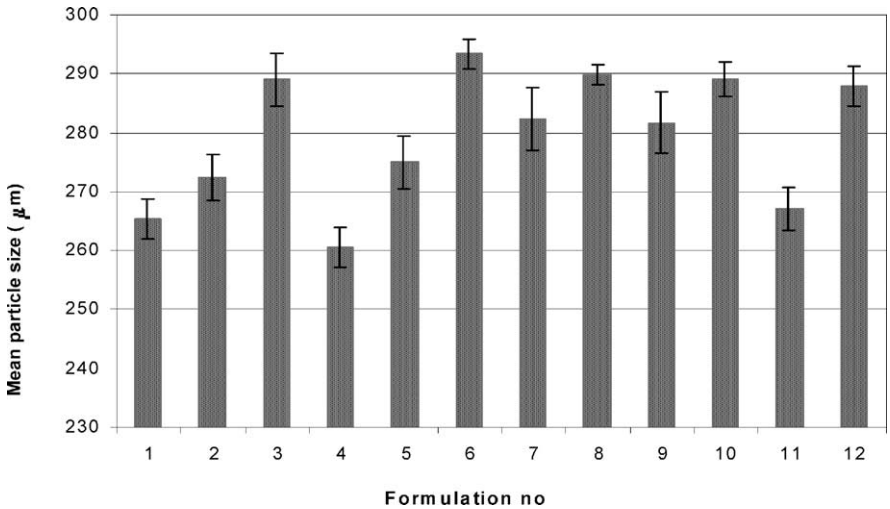

Fig. 4. Mean particle sizes of the microspheres ($n = 3$).

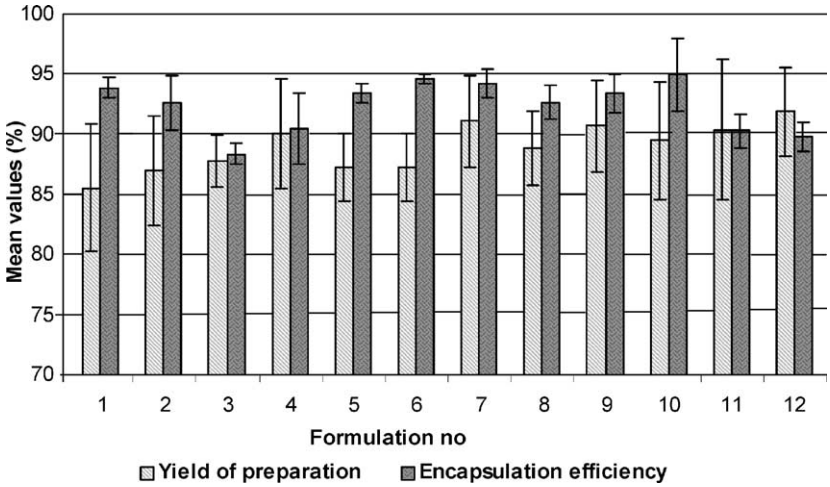

Fig. 5. Yield of preparation and encapsulation efficiency data ($n = 3$).

Akbuğa (1989) also reported that the particle size distributions were not affected by the type of polymer for acrylic polymer microspheres.

The yields of preparation and acetazolamide encapsulation efficiencies were very high for all microspheres obtained and were not affected by the type of polymer ($P > 0.05$), the polymer:drug ratio ($P > 0.05$), the stirring speed of the system ($P > 0.05$) and the ratio of the mixture of polymers ($P > 0.05$). The yields of preparation and encapsulation efficiencies are shown in Fig. 5.

The equilibrium solubilities of acetazolamide in pH 1.2 and pH 7.4 buffer solutions were found to be 1.23 ± 0.16 mg/ml and 2.43 ± 0.42 mg/ml, respectively, indicating the sink condition limits for the dissolution studies.

The dissolution rate of pure drug and the drug release rate from the microspheres were studied at pH 1.2 and pH 7.4 using the USP XXIII paddle method. Dissolution rate of acetazolamide was pH-dependent as shown in Fig. 6. Acetazolamide is a weak acid drug (The Merck Index, 2001) and its solubility is higher at high pH, as expected.

Acetazolamide release rate from microspheres was dependent on the type of polymer used as shown in Figs. 7–10. In order to keep the total surface area of the microsphere samples constant and thus to get comparable results, the release studies all were carried out

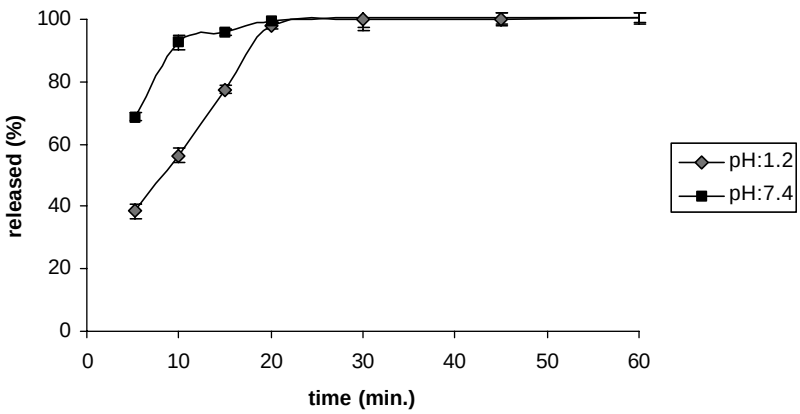

Fig. 6. Dissolution profiles of pure acetazolamide in pH 1.2 and pH 7.4 buffer solutions ($n = 3$).

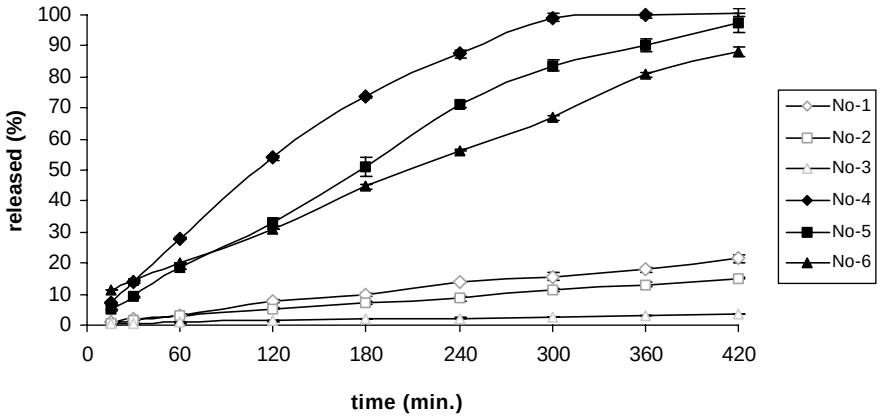

Fig. 7. In vitro release of acetazolamide from Eudragit RS (open symbols) and Eudragit RL (closed symbols) microspheres in pH 1.2 buffer solution ($n = 3$).

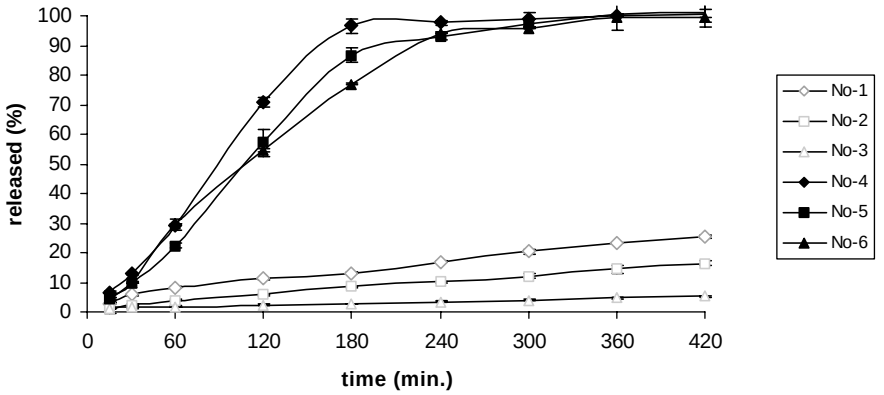

Fig. 8. In vitro release of acetazolamide from Eudragit RS (open symbols) and Eudragit RL (closed symbols) microspheres in pH 7.4 buffer solution ($n = 3$).

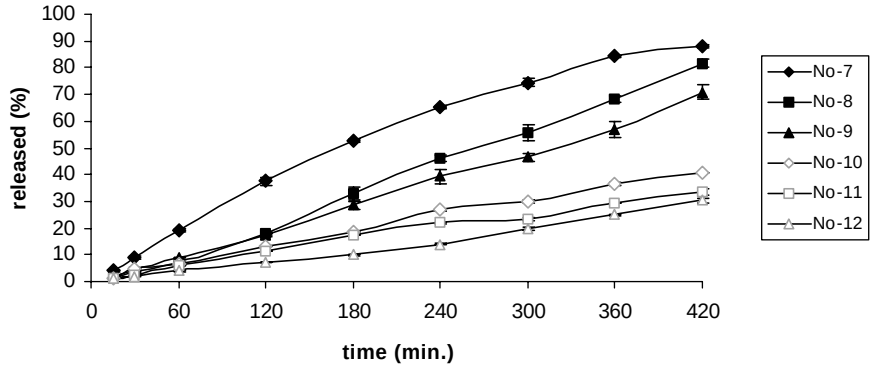

Fig. 9. In vitro release of acetazolamide from Eudragit RS/Eudragit RL microspheres in pH 1.2 buffer solution ($n = 3$) (closed symbols, polymer:drug ratio 2:1, stirring speed 500 rpm and open symbols, polymer:drug ratio 3:1, stirring speed 750 rpm).

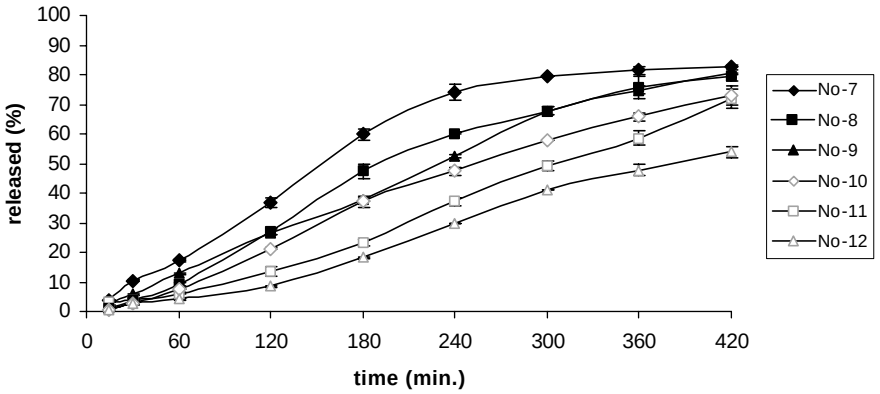

Fig. 10. In vitro release of acetazolamide from Eudragit RS/Eudragit RL microspheres in pH 7.4 buffer solution ($n = 3$) (closed symbols, polymer:drug ratio 2:1, stirring speed 500 rpm and open symbols, polymer:drug ratio 3:1, stirring speed 750 rpm).

with 250 μm size fractions of the microspheres prepared.

Acetazolamide release rate from Eudragit RS microspheres was very slow and incomplete for all formulations (Figs. 7 and 8; formulations nos.: 1–3) at both pH values. Dissolution study was carried out for 24 h for formulation 1 at pH 7.4, then microspheres were collected by filtering the dissolution medium, dried, powdered and acetazolamide was assayed. This recovery study confirmed that most of the drug was still present in the microspheres. Armand et al. (1987) reported that a large part of sodium salicylate remained in a spherical matrix of Eudragit RS even after 20 h exposure to dissolution medium. They suggested an interaction between the polymer and the drug. Vachon and Nairn (1995) also prepared Eudragit RS microspheres containing acetylsalicylic acid as a model drug and investigated drug release rate from formulations having different polymer:drug ratios. They reported that drug release was incomplete from all formulations tested and that the cumulative amount of drug released after 24 h was 60%. Remaining of the drug in microspheres was also explained by the interaction between drugs containing carboxylic acid functional groups and the polymer.

As drug release rates were very slow and incomplete from Eudragit RS microspheres, the same formulations were prepared using Eudragit RL as polymer and different drug release profiles were observed. Acetazolamide release rates from Eudragit RL microspheres were fast and the amount released in 7 h reached 100% for some formulations. This is due to the fact, that the amount of quaternary ammonium groups of Eudragit RS is lower than that of Eudragit RL, therefore, Eudragit RL is more permeable to water, so that release is less retarded. Similar results were reported by Akbuğa (1989); Kawashima et al. (1989).

Drug release rates from Eudragit RS and Eudragit RL microspheres were affected by polymer:drug ratio and stirring speed of the system. The release rates of acetazolamide decreased as polymer:drug ratio increased. The difference was significant for 7 h with Eudragit RS microspheres ($P < 0.01$) and for 5 h with Eudragit RL microspheres ($P < 0.01$) in pH 7.4 (Fig. 8). The difference was also significant ($P < 0.01$) for 7 h with both polymers in pH 1.2 (Fig. 7). Pongpaibul et al. (1984) reported that the effect of polymer:drug ratio on the release rate was not significant for Eudragit microspheres prepared by an emulsion-solvent evaporation method in contrary to the results of our study.

Increasing the stirring speed of the system decreased the mean particle size as mentioned before and this led to an increase of release rate as would expected from surface area relationship. Similar results were reported by Pongpaibul et al. (1984). The difference was significant for 7 h with Eudragit RS microspheres ($P < 0.01$) and for 5 h with Eudragit RL microspheres ($P < 0.01$) as shown (Fig. 8) in pH 7.4. The difference was also significant for 7 h with both polymers in pH 1.2 (Fig. 7).

Table 2
Correlation coefficients of different mathematical models for acetazolamide microspheres

No.	First-order	Higuchi	Modified Hixon Crowell	Zero-order
1	0.9904	0.9824	0.9891	0.9857
2	0.9980	0.9806	0.9977	0.9967
3	0.9987	0.9677	0.9987	0.9988
4	0.9545	0.9438	0.9377	0.8736
5	0.9817	0.9703	0.9863	0.9304
6	0.9558	0.9760	0.9900	0.9144
7	0.9633	0.9671	0.9495	0.9094
8	0.9823	0.9795	0.9925	0.9823
9	0.9081	0.9019	0.9397	0.9800
10	0.9945	0.9820	0.9899	0.9618
11	0.9942	0.9827	0.9980	0.9827
12	0.9786	0.9281	0.9786	0.9851

By examining the release profiles from Eudragit RL microspheres in detail (Fig. 8; formulations nos.: 4–6), it can be seen that the rates were relatively fast and that more than 70% of acetazolamide was released in 3 h at pH 7.4.

Davis (1985) reported that the gastrointestinal transit time is 6–8 h for young healthy men. Considering the gastrointestinal transit time, release profiles from Eudragit RL microspheres seem to be too fast for controlled release. To decrease the release rate Eudragit RL and Eudragit RS were mixed at different amounts for two microsphere formulations. Acetazolamide dissolution rates from these microspheres are shown in Figs. 9 and 10. The amount of acetazolamide released in 7 h was between 54 and 82% for different formulations. Release rates decreased as the amount of Eudragit RS increased and the difference was significant ($P < 0.01$) for all formulations as shown in Figs. 9 and 10.

Correlation coefficients of different mathematical models shown in Table 2 were obtained using the software developed by Ege et al. (2001).

For the first three formulations, a single polymer, Eudragit RS was used and the release rate data were found to fit first-order kinetics. When Eudragit RL was used as polymer, correlation coefficients nearly for all formulations fitted first-order kinetics.

When Eudragit RS and Eudragit RL were used in combination, zero-order release kinetics provides the best correlation until 5 h nearly for all formulations.

4. Conclusion

Acetazolamide microspheres were prepared successfully using solvent evaporation method. Polymer:drug ratio and stirring speed of the system were important to obtain spherical particles with smooth surfaces. The yields of preparation and encapsulation efficiencies were very high for all microspheres obtained. Acetazolamide release rates from microspheres were dependent on the type of polymer used. Acetazolamide release rates from Eudragit RS microspheres were very slow whereas release rates from Eudragit RL microspheres were faster. The drug release profile aimed for peroral administration could be obtained by adding Eudragit RS to Eudragit RL and changing the ratio of these polymers. Controlled release without initial peak levels achieved with these microsphere formulations can reduce dosing frequency, decrease side effects and improve patient compliance.

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References

Akbuğa, J., 1989. Preparation and evaluation of controlled release furosemide microspheres by spherical crystallization. Int. J. Pharm. 53, 99–105.

Armand, J.Y., Magnard, F., Bouzon, J., Rollet, J., Taverdet, J.L., Vergnaut, J.M., 1987. Modelling of drug release in gastric liquid from spheric galenic forms with Eudragit matrix. Int. J. Pharm. 40, 33–41.

Arshady, R., 1990. Albumin microspheres and microcapsules: methodology of manufacturing techniques. J. Control. Release 14, 111–131.

Arshady, R., 1991. Preparation of biodegradable microspheres and microcapsules 2. Polylactides and related polyesters. J. Control. Release 17, 1–22.

Babay, D., Hoffman, A., Benita, S., 1988. Design and release kinetic pattern evaluation of indomethasin microspheres intended for oral administration. Biomaterials 9, 482–488.

Davis, S.S., 1985. The design and evaluation of controlled release systems for the gastrointestinal tract. J. Control. Release 2, 27–38.

Davis, S.S., Illum, L., 1988. Polymeric microspheres as drug carriers. Biomaterials 9, 111–115.

Ege, M.A., Karasulu, H.Y., Karasulu, E., Ertan, G., 2001. A computer program designed for in vitro dissolution kinetics; in vitro–in vivo kinetic correlations and routine applications. In: Proceedings of the 4th Central European Symposium on Pharmaceutical Technology. Vienna; Scientia Pharmaceutica Suppl. 1, Band 69, pp. S127–S128.

El-Gazayerly, O.N., Hikal, A.H., 1997. Preparation and evaluation of acetazolamide liposomes as an ocular delivery system. Int. J. Pharm. 158, 121–127.

Eudragit RS and Eudragit RL data sheets, 1991. Röhm Pharma GmbH, Darmstadt.

Garner, L.L., Carl, E.F., Ferwerda, J.R., 1963. Advantages of sustained release therapy with acetazolamide in glaucoma. Am. J. Ophthalmol. 55, 323–327.

Kawashima, Y., Niwa, T., Handa, T., Takeuchi, H., Iwamoto, T., Itoh, K., 1989. Preparation of controlled release microspheres of ibuprofen with acrylic polymers by a novel quasi-emulsion solvent diffusion method. J. Pharm. Sci. 78, 68–72.

Martindale, 2001. In: Parfitt, K. (Ed.), The Complete Drug Reference, 32nd ed. Martindale Pharmaceutical Press, London, p. 811.

Pongpaibul, Y., Price, J.C., Whitworth, C.W., 1984. Preparation and evaluation of controlled release indomethacin microspheres. Drug Dev. Ind. Pharm. 10, 1597–1616.

Pongpaibul, Y., Maruyama, K., Iwatsuru, M., 1988. Formation and in vitro evaluation of theophylline-loaded poly(methyl methacrylate) microspheres. J. Pharm. Pharmacol. 40, 530–533.

Ritschel, W.A., 1989. Biopharmaceutic and pharmacokinetic aspects in the design of controlled release peroral drug delivery systems. Drug Dev. Ind. Pharm. 15, 1073–1103.

Tsankov, S.T., Lambov, N., Minkov, E., 1992. Regulation of the tetracycline hydrochloride release from polyacrylate microspheres. Pharmazie 47, 125–128.

The Merck Index, 2001, 13th ed. In: Maryadele, J.O'Neil (Ed.), Merck&Co. Inc., Whitehouse Station, NJ, p. 56.

US Pharmacopoeia XXIII, 1995. US Pharmacopoeial Convention Inc., Rockville, MD.

Vachon, M.G., Nairn, J.G., 1995. Physico-chemical evaluation of acetylsalicylic acid—Eudragit RS 100 microspheres prepared using a solvent-partition method. J. Microencapsul. 12, 287–305.