International Journal of Pharmaceutics, 66 (1990) 29-37
Elsevier

IJP 02228

Drug release from submicronized o/w emulsion: a new in vitro kinetic evaluation model

M.Y. Levy and S. Benita

Department of Pharmacy, The Hebrew University of Jerusalem, Jerusalem (Israel)

(Received 13 March 1990)
(Modified version received 14 June 1990)
(Accepted 30 June 1990)

Key words: Submicron emulsion; Drug release; Membrane diffusion; Dialysis diffusion technique; Reverse dialysis; Diazepam

Summary

The in vitro release of diazepam from a submicron o/w emulsion was evaluated using the dialysis bag technique diffusion and the bulk-equilibrium reverse dialysis bag technique. Irrespective of the nature of the sink solution used, the release rate of diazepam from different emulsion dosage forms remained slow and incomplete as compared to a diazepam hydroalcoholic solution using the dialysis bag technique. This was attributed to a marked decrease in the aqueous drug gradient of drug available for membrane diffusion in the presence of the oily internal phase, rendering the permeation through the dialysis membrane the rate-limiting step in the overall kinetic process. It can definitely be deduced that the dialysis bag technique could not be considered an appropriate method to evaluate the true release mechanism of a drug from a colloidal carrier. An in vitro kinetic model is therefore proposed where the colloidal drug carrier suspension is directly placed in the release solution and has the opportunity to release the drug content under maximum dilution and perfect sink conditions. The drug released sampling is performed through immersed dialysis bags previously filled and equilibrated with the sink solution in the receptor compartment. The release profiles of diazepam from the actual submicron emulsion was similar to that observed from marketed aqueous and emulsion dosage forms correlating well with pharmacokinetic results reported in the literature. It was found that the release rate from the oily nanodroplets was faster than the permeation rate through the dialysis membrane which should be the slowest step governing the overall kinetic process despite rapid and complete diffusion of dissolved drug within less than 1 h. In view of the overall results it can be concluded that the release of diazepam from submicron emulsion is very rapid under perfect sink conditions.

Introduction

The characterization of in vitro drug release from a colloidal carrier, especially under sink conditions, is technically difficult to achieve. This should be attributed to the inability to separate effectively the particles from the dissolved or released drug in the sink solution owing to the very small size of the dosage form particles. Attempts were made to evaluate drug release using various techniques such as cell diffusions (Friedman and Benita, 1987; Lostritto et al., 1987) where the undiluted colloidal drug carrier suspension was separated from the sink solution by polycarbonate porous membrane or dialysis membrane. Other investigators attempted to examine the drug release using the dialysis bag technique under either dynamic or static conditions (Sasaki et al., 1984; Gupta et al., 1987; Ammoury et al., 1989).

The dialysis bag technique was recently criticized by Washington (1989) who claimed that since

Correspondence: S. Benita, School of Pharmacy, Dept. of Pharmacy, P.O. Box 12065, Jerusalem 91120, Israel.

the carrier suspension is never diluted, the experiment is not performed under sink conditions even if such conditions are constantly maintained in the acceptor compartment where sampling is performed. Consequently, the method does not measure the true release rate but rather the partition of a drug between the various phases of a dispersed system. Although mathematical attempts (Friedman and Benita, 1987; Gupta et al., 1987; Lostritto et al., 1987) have been made taking into consideration the different constraints of such a system, it appears that the membrane diffusion technique has limited if any, useful application in predicting in vivo behaviour of colloidal drug carriers intended for administration directly into the blood stream (i.v.) or to sites where perfect biological sink conditions prevail (p.o.). Furthermore, the appearance rate of the drug in the sampling compartment (out of the dialysis membrane) was reported to be dependent on experimental factors (drug-excipient interactions, micelles, osmosis, etc.) which are most of the time difficult to keep constant (Ammoury et al., 1990).

Recently, the physicochemical properties of a submicronized o/w emulsion of diazepam for parenteral use were reported (Levy and Benita, 1989). In the present study, it is intended to propose a new in vitro release experimental model able to predict in vivo behaviour of a drug released from a colloidal carrier by applying this model to the various diazepam submicron emulsions. In addition, an in vitro drug release kinetic comparison was carried out between the new experimental model and the widely used dialysis bag technique.

Materials and Methods

Materials

Dialysis tubes (Spectra/por 4, diameter 25 mm, m.w. cutoff: 12000–14000 or Spectra/por 6, diameter 28 mm, m.w. cutoff: 50000) and appropriate closures were purchased from Spectrum Inc., Los Angeles, U.S.A. Human serum albumin (HSA) solution was furnished by Kamapharm, Ltd, Kibbutz Beit Kama, Israel. Hepes buffer was supplied by Sigma, St. Louis, U.S.A. The marketed diazepam injections (Assival^R, equivalent to Valium^R injections, manufactured by Teva Industries, Kfar Saba, Israel, under license from Hoffman La Roche, Basel, Switzerland) were purchased from a hospital pharmacy, batch no. 0983. The marketed diazepam submicron emulsion, Diazemuls^R was manufactured by Kabi Vitrum, Stockholm, Sweden, batch no. 68107-01.

Methods

The materials and methods used to prepare the diazepam submicron emulsion were reported elsewhere (Levy and Benita, 1989).

In vitro release kinetic experiments

Diazepam solubility experiments. The solubility of diazepam in the release solution was measured using a technique described elsewhere (Levy and Benita, 1989). The solubility of diazepam in water and Hepes buffer pH 7.0 was 0.035 and 0.05 mg/ml, respectively, at room temperature. These results conformed with those already reported by other authors (Mura et al., 1988).

The solubility of diazepam increased with the increased concentration of albumin, reaching a value of 0.25 mg/ml at 1% HSA solution, confirming previous results reported in the literature (Olson and Faith, 1988). Therefore, there is no need for more concentrated albumin solutions and the 1% albumin solution would be sufficient to guarantee sink conditions.

Dialysis bag diffusion technique. 1 ml of the diazepam submicron emulsion was placed in the dialysis bag, hermetically sealed and dropped into 1 l of the sink solution. The entire system was kept at 37°C with continuous magnetic stirring of the sink solution. The kinetic experiments were carried out with various aqueous solutions respecting sink conditions in the receptor compartment (water, alcohol 70%, albumin 1%). Samples (1 ml) were withdrawn from the receptor compartment at predetermined time intervals and assayed for diazepam content by a modified HPLC technique previously reported (Klockowski and Levy, 1987). Calibration curves of diazepam were constructed in either water, Hepes buffer pH 7.0 and 1% albumin solutions. Peaks of diazepam were

sharp and clearly separated from those of carbamazepine (internal standard). The standard curves yielded linear relationships ($r^2 = 0.997$) when the ratio of the peak area of drug standard to that of the internal standard was plotted as a function of known concentrations of diazepam (at least in the range tested from 1 to 8 μg/ml irrespective of the sink solutions used). The average diazepam extraction yield calculated according to Klockowski and Levy (1987) was found to be higher than 95%. Similar behaviour was noted in all the sink solutions tested and the assay procedure was highly reproducible (each sample was analysed in triplicate).

Bulk-equilibrium reverse dialysis bag technique. The diazepam submicronized emulsion (0.3 ml) was directly placed into 500 ml of a stirred sink solution where numerous dialysis sacs containing 1 ml of the same sink solution were previously immersed. It should be emphasized that the dialysis sacs were equilibrated with the sink solutions for a few hours prior to experiments. It was important to check that the various release solutions such as water, 1 mM Hepes buffer pH 7.4 and 1% albumin solution would not alter the physical integrity of the emulsions tested. It has already been reported that Hepes buffer, which has a low ionic strength, does not affect the physicochemical properties of fat emulsions (Washington et al., 1989). Nevertheless, no difference in the particle distribution profile was noted before and after considerable dilution in the various sink solutions using a PCS technique (Levy and Benita, 1989). Since the release of diazepam from the emulsion could not be followed directly in the sink solution without separating the solution from the tiny oily droplets, at predetermined time intervals, a dialysis bag is withdrawn from the stirred release solution, and the content of the dialysis bag is assayed for diazepam by HPLC.

The dialysis membranes were selected following the screening of various types of membranes. It was found that no diazepam adsorption occurred and the membrane was freely permeable to the active ingredient at two different m.w. cutoffs: 12000–14000 and 50000.

The kinetic experiments were performed at a fixed temperature of 37°C under constant magnetic stirring.

Chromatographic conditions. A Milton Roy HPLC (Model Constametric 3000) equipped with a variable-wavelength ultraviolet detector (Spector Monitor 3100, Milton Roy), a Milton Roy Integrator, and a 25 cm × 4.6 mm i.d. reverse-phase column, Econosil C-18 10 μm (Altech Association, Inc, Deerfield, IL, U.S.A.) were used. The column was eluted with acetonitrile-methanol-water (44:12:44 by vol.) at a rate of 1.5 ml/min, and the column eluent was monitored at 242 nm. The chromatograph was operated at a pressure of 2200 p.s.i. at room temperature.

Results and Discussion

Dialysis bag diffusion technique

The kinetic model proposed is based on previous cited studies which examined the release of the drug from a colloidal carrier suspension enclosed in a small membrane-enveloped compartment as schematically presented in Fig. 1 (I).

Diazepam is freely soluble in 70% alcohol solution. It can be seen from Fig. 2 (upper graph) that diazepam at an initial concentration of 5 mg/ml (Assival^R) in the dialysis sac diffused out rapidly into the sink solution which consisted of 70% alcohol. It should be noted that the properties of the various dialysis membranes are not affected by 70% alcohol. These results clearly indicate that the membrane is not a rate-limiting factor under the experimental conditions used where a given large diazepam gradient was established between the internal and external compartments separated by the membrane. However, when the same marketed hydro-alcoholic solution is released in the same volume of water, the release rate is drastically reduced (Fig. 2). This can be explained by the fact that water penetrated rapidly into the dialysis sac concomitantly with diffusion out of the alcohol resulting in a rapid decrease in the alcohol concentration in the sac which led to diazepam precipitation inside the dialysis sac. Furthermore, the diazepam release rate was much slower from both Diazemuls^R and the actual diazepam emulsion, reflecting the inability of the emulsions to release even a small fraction of the drug over a 30 h period (Fig. 2). Addition of 1% albumin to the

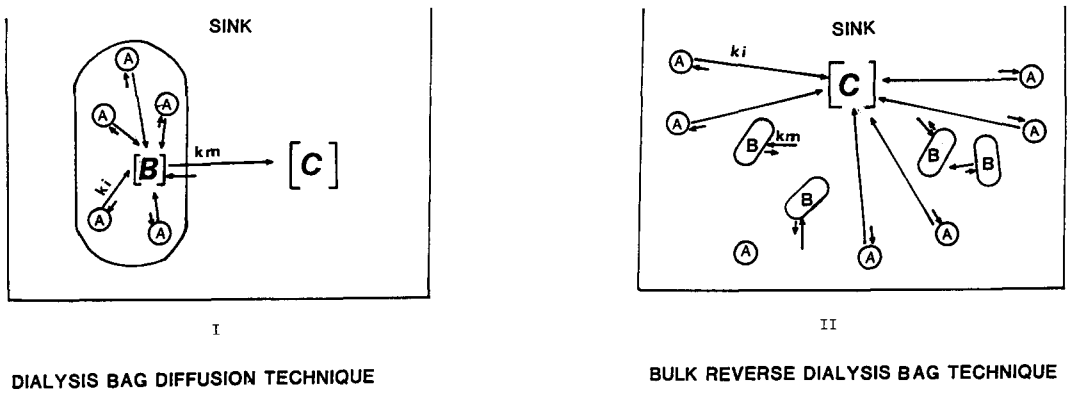

Fig. 1. Schematic illustration of the dialysis bag diffusion technique (I) and the bulk-equilibrium reverse dialysis bag technique (II) where drug concentration is represented by A in the oily droplets, B in the internal aqueous phase of the dialysis bags and C in the sink solution.

sink solution and increase of the m.w. cutoff of the dialysis membrane enhanced the diazepam release from both emulsions (Fig. 3) but release rates remained slow and incomplete. However, no precipitation of diazepam from the hydroalcoholic solution was detected in the presence of 1% albumin resulting in rapid release as expected (Fig. 3). Nevertheless, the kinetic behaviour of both emulsions was similar despite the constraints. Numerous investigators have pointed out various reasons

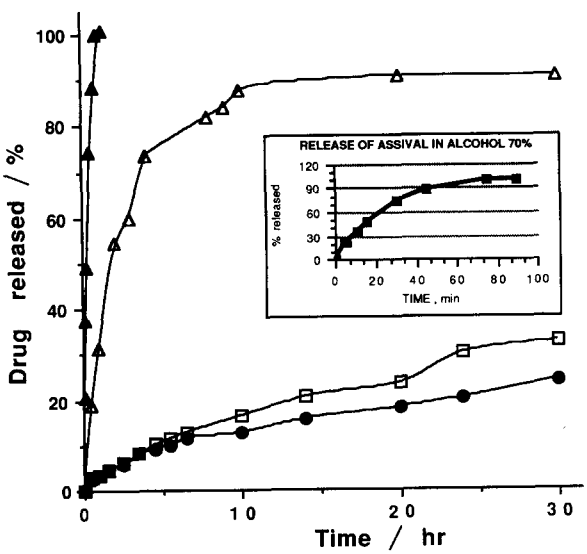

Fig. 2. Diazepam release profile from the various dosage forms as a function of sink solution nature using the dialysis bag technique (m.w. cut off 12000–14000). (▲) Assival in 70% alcohol; (△) Assival in water; (□) Diazemuls in water; (●) diazepam emulsion in water.

explaining the drastic decrease in release rate of a drug from the emulsion using the dialysis technique (Lostritto et al., 1987; Washington, 1989; Ammoury et al., 1990). The drug partition in favour of the oily nanodroplets and other potential existing colloidal structures, such as bilayers or large micelles which reduced markedly the aqueous drug concentration available for diffusion through the dialysis membrane, should be considered the main factor contributing to the marked decrease in release rate of the drug from the emulsion in the dialysis sac. In most of the kinetic studies performed to evaluate drug release from colloidal carriers, it was assumed that drug diffusion through the dialysis membrane was not the rate-limiting step in the overall kinetic process. Such an assumption was probably not verified in most of the studies performed, since no evaluation of drug diffusion through dialysis membrane under very low drug gradient was carried out, probably owing to a lack of accurate and sensitive analytic methods able to estimate quantitatively very low drug concentrations in the receptor compartment.

Another factor which probably plays an important role and is mostly neglected is the surface area available for drug diffusion. In the present study, the surface area of the dialysis sac in direct contact with the dissolved drug in the external phase of the emulsion available for diffusion is 6.33 cm^2, while the interfacial area regenerated by the dispersed oily nanodroplets is 36000 cm^2. It should be taken into consideration that the first-

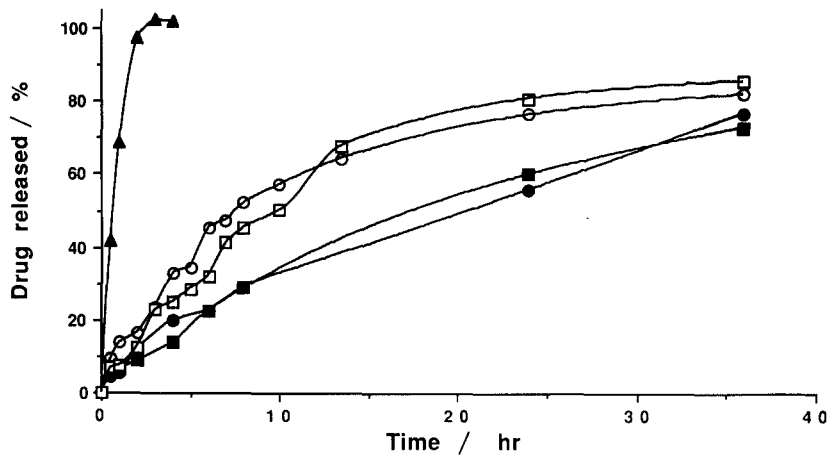

Fig. 3. Diazepam release profile from various dosage forms in 1% human serum albumin solution as a function of the membrane dialysis m.w. cut off using the dialysis bag technique. (▲) Assival in 12000–14000 cut off; (●) diazepam emulsion in 12000–14000 cut off; (■) Diazemuls in 12000–14000 cut off; (○) diazepam emulsion in 50000 cut off; (□) Diazemuls in 50000 cut off.

order release constant, k, comprises AD/h where A is the surface area, D the coefficient diffusion and h the thickness, the subscripts m, i, referring to the dialysis membrane or the interfacial complex film emulgator respectively. It is therefore believed that the dialysis membrane should be the rate-limiting factor in the overall kinetic process even if D_m is much larger than D_i, since A_i is much larger than A_m and h_i is much smaller than h_m rendering the value of k_m smaller than k_i. In view of the overall results reported it can be definitely deduced that the dialysis sac technique could not be considered an appropriate method to evaluate the true release rate of a drug from a colloidal carrier.

Bulk-equilibrium reverse dialysis bag technique

The current kinetic model proposed is based on a new concept where the colloidal drug carrier suspension is directly placed in the release solution where it has the opportunity to release the drug load under maximum dilution (perfect sink conditions). It would be very difficult to assay the drug release in the presence of the colloidal carrier since no efficient technical method is available to separate the colloidal carrier from the dissolved or release drug. In the present work, an attempt was made to overcome the obstacle by immersing numerous dialysis bags previously filled and equilibrated with the sink solution in the receptor compartment as shown in Fig. 1 (II). In the undiluted submicron emulsion, the drug is partitioned between the oily and aqueous phases according to the drug solubility. At t=0, as the colloidal dispersion is diluted with the sink solution, a new equilibrium is re-established and drug partitioned between the oily nanodroplets and the sink solution which became the external phase of the colloidal dispersion without being separated from the oily droplets by any artificial membrane. Therefore, the oily nanodroplets displaying a considerable large interfacial area are directly exposed to the extended sink volume solution reflecting a natural biological environment. Drug will therefore diffuse out from the oily droplets to the sink solution according to a true and real gradient existing between the oily and the regenerated apparent aqueous phase. Drug dissolved in the aqueous phase will easily diffuse through the sink solution into the dialysis bags. It should be noted that only dissolved drug and other small molecules present in the system will penetrate the dialysis membranes and that drug diffusion will obey Fick's first law. The small solute molecules including the drug freely pass through the membrane until equilibrium is reached between the internal and external aqueous solutions. The actual method is a modification of the well-known technique called reverse dialysis which is normally used to concentrate ma-

terial in the bag. In the present case, in order to prevent any perturbation of the equilibrium between the various phases present in the entire kinetic system, it was decided to allow drug to diffuse in the bag only according to a natural gradient until an equilibrium had been reached. It should be emphasized that the total volume of the dialysis bags (10–15 ml) is much smaller than the volume of the sink solution (500 ml). Therefore, the concentration of the drug in the sink solution is not affected by the diffusion of the drug in the dialysis bag as confirmed by concomitant sampling from the sink solution and the dialysis bags. It was observed that the drug concentration increased in the bag while it remained practically constant in the sink solution. The percent drug release was calculated from the ratio of drug concentration measured at predetermined time intervals in the dialysis bags vs the total concentration of the drug in the sink solution where oily nanodroplets are also present. Dialysis membrane control of drug diffusion would be expected to manifest a permeation rate depending on a changing concentration gradient throughout the process. The effect of initial drug concentration in the sink solution or permeation rate should therefore be addressed. The permeation rate of various initial concentrations of diazepam (ranging from 10^{-5} to 10^{-6} M) from the sink solution into the dialysis bags was evaluated. Assuming that drug diffusion through all the dialysis bags was identical at the same time point, no difference in the various diffusion profiles was noted in the range of drug concentrations tested. These results indicated that decreasing the initial concentration gradient by a factor of 10 did not affect the permeation rate of the drug which remained rapid, suggesting that the drug freely diffused into the dialysis bags. It should be noted that according to the experimental conditions used, the maximum drug released in the sink solution should be 10^{-5} M. It was not possible to estimate accurately less than 10^{-6} M diazepam by the HPLC method proposed. Therefore, it was not possible to evaluate the potential effect of much smaller drug concentration gradients on the drug permeation profile in the dialysis bags.

The release profile of diazepam from the submicron emulsion was similar to that observed from the marketed diazepam solution (Fig. 4A). For purposes of clarification and convenience, the drug permeation profile was rather defined as the drug release profile although it does not reflect the real release profile of the drug in the sink solution. Despite the marked increase in the solubility of diazepam in the presence of albumin, no marked change in the diazepam release profile from the submicron emulsion was noted by varying the nature of the sink solution (Fig. 4A and B), suggesting the prevalence of perfect sink conditions throughout the entire kinetic process irrespective of the nature of the release solution. These results

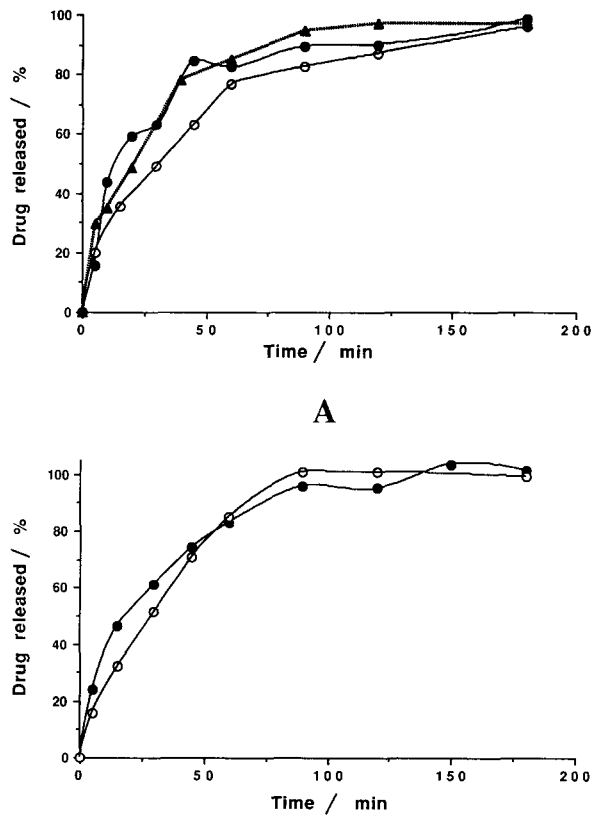

Fig. 4. Diazepam release profile from a submicron emulsion using the bulk equilibrium reverse dialysis bag (m.w. cut off 12000–14000) technique as a function of the sink solution nature: (A) water or 1% HSA solution and (B) Hepes buffer pH 7.0 or buffered 1% HSA solution. A: (•) Diazepam emulsion in water; (○) diazepam emulsion in 1% HSA solution; (▲) Assival in water. B: (•) Diazepam emulsion in buffer; (○) diazepam emulsion in 1% buffered albumin solution.

were further confirmed by the observed similarity in the release profiles of diazepam from marketed aqueous and emulsion dosage forms (Assival^R and Diazemuls^R, respectively) and from the actual emulsion (Fig. 5). Such kinetic behaviour correlates very well with the pharmacokinetic results published by Von Dardel and colleagues (1983) who reported that the distribution and elimination phases after i.v. injection to patients were practically the same with Diazemuls^R and Valium^R dosage forms.

The increase in the m.w. cut off from 12000–14000 to 50000 did not alter the permeation or release rate of the diazepam from the various dosage forms, indicating that the small drug molecule diffused freely through both dialysis membranes. As expected, the diazepam release profile decreased markedly with decreasing sink conditions in the release solution where total diazepam concentration could reach sub-saturation levels from 5 to 100% (Fig. 6). It should be emphasized that the sub-saturation levels were calculated on the basis of diazepam solubility in water at room temperature. The sub-saturation values reported in the legend of Fig. 6 should only be considered as approximations and the real sub-saturation levels should take into consideration the temperature increase, the presence of the oily phase and the various components of the emulsion. The maximum amount released and release rate declined with increasing sub-saturation levels that could be reached by the total diazepam concentration released as a result of drug partitioning in favour of the larger oily phase present in the system. This is confirmed by the lack of influence on drug release profile in the kinetic experiment where total diazepam concentration released could reach sub-saturation level of 20%. Indeed, the actual sub-saturation level should be smaller and therefore remains within the limits of the sink conditions which do not disturb the free release of a drug from the dos-

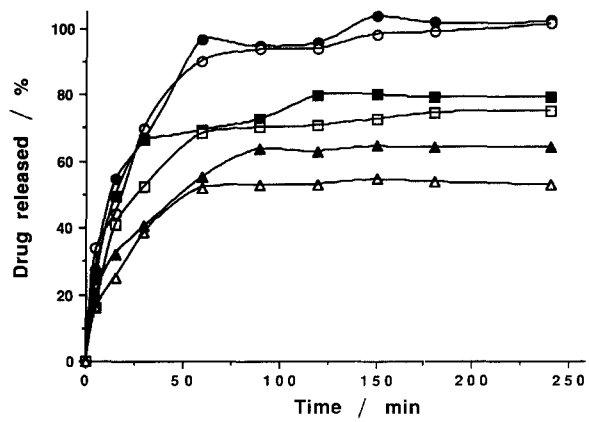

Fig. 6. Diazepam release profile from the submicron emulsion as a function of various diazepam saturation levels in the sink solution (same technique as in Fig. 4). (●) SC 5%; (○) SC 20%; (■) SC 40%; (□) SC 60%; (▲) SC 80%; (△) SC 100%.

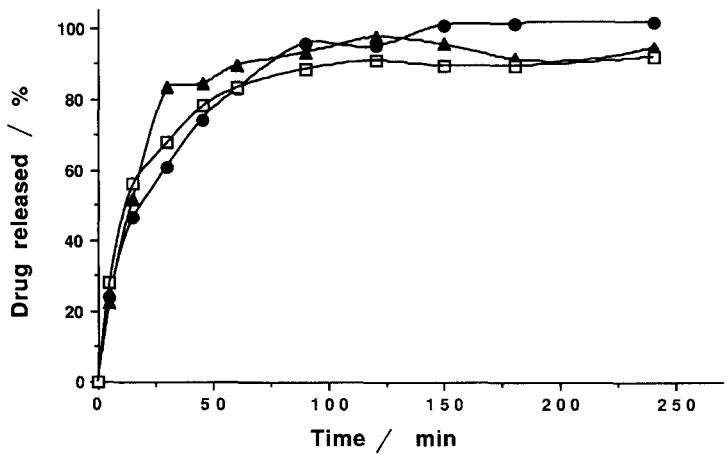

Fig. 5. Diazepam release profile from various diazepam dosage forms in hepes buffer pH 7.0 sink solution (same technique as in Fig. 4). (▲) Assival; (●) diazepam emulsion; (□) Diazemuls.

age form. It can be deduced that the kinetic release process is very sensitive to the prevalence of sink conditions. Any factor which will perturb such conditions will alter the release profile from the emulsion. Furthermore, the kinetic behaviour of diazepam release from the emulsions was not at all altered by the increase in the oily phase volume ratio while the amount of diazepam was kept constant, nor was it changed by the variation in pH of the sink solution from 6 to 8.

In view of the overall results achieved in this part of the study, it can be deduced that the release of diazepam from the submicron emulsions into sink solutions is rapid. The release from the oily droplets is faster than the permeation rate through the dialysis membrane which should be the slowest step and consequently the rate-determining step in the overall kinetic process. There is no doubt that the kinetic system proposed is able to differentiate between colloidal drug delivery carriers releasing their content over a period greater than 1 h. However, in this particular case, the minor differences which should exist between the various diazepam dosage forms were not clearly detected by the reverse dialysis technique. Therefore, an attempt to derive an exact mathematical solution with regard to drug release from the submicron emulsion appears impossible. This would require an accurate knowledge of the release kinetic pattern followed by diazepam in the emulsion. Such information is not yet available as a result of dialysis membrane diffusion limitation. The search for more freely permeable membranes should be carried out to allow rapid and complete drug diffusion inside the dialysis bag within minutes from the start of the kinetic experiment.

Conclusion

Since systematic experimental data on interfacial film or partition rate-control from the emulsion are not available and a direct quantitative evaluation is not possible at this stage of the investigation, only a qualitative conclusion can be derived from the analysis of the kinetic results. It can be concluded that the release of diazepam from submicron emulsions under sink conditions is rapid. The kinetic process is probably not controlled by the oil-water partition rate of the emulsion but rather by the diffusion of the drug through the interfacial co-emulgator film. This suggestion needs to be confirmed by an independent kinetic study where the dialysis bags are replaced by adequate inert, unloaded vesicular microstructures. These would markedly increase the interfacial membrane area and would therefore respond faster to drug diffusion.

Acknowledgement

The authors would like to acknowledge the support of this study by Avitek Ltd, Rehovot, Israel.

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