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Drug dissolution studies in milk using the automated flow injection serial dynamic dialysis technique

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Summary

The application of flow injection serial dynamic dialysis (FISDD) technique to monitor dissolution studies in complex media is described. The method is based on the study of the kinetics of the simultaneous dissolution and dialysis processes. Commercial formulations of salicylamide, propantheline bromide, nitrofurantoin and acetaminophen were used to investigate the utility of the FISDD technique for studying the dissolution of drugs in low fat milk. The latter was utilized as a food simulating medium. Dissolution studies were also conducted in phosphate buffer of pH 6.5. In each case the FISDD system was coupled with the rotating basket apparatus. The determination of the dialyzable drugs was performed automatically by the FIA analyzer. A fully automated monitoring of dissolution of drugs in milk and buffer was achieved. In all cases the dissolution rate of drugs in milk was lower than the corresponding rate in the aqueous buffer. The potential significance of this system with respect to the in vitro study of dissolution of drugs in food simulating media is discussed. This system could be used either to reveal or explore food–drug and/or food–formulation interactions anticipated or observed in vivo.

Introduction

In vitro dissolution procedures have been developed in order to assess drug dissolution behaviour under conditions that simulate, at least in part, the conditions in vivo. Hence, the composition of fluid into which dissolution occurs plays a primary role in a dissolution test system. Aqueous systems containing a buffer, dilute hydrochloric acid, or simply pure water are the most common but surfactants, cosolvents, or a sequence of gastric and intestinal fluid have been employed (USP XXI, 1985). Frequently, the composition of the dissolution medium is more complex. This happens when the dissolution characteristics of drugs are evaluated in media containing macromolecules or from appropriate formulations of drug-macromolecule. Typical examples of such additives or formulations are, polyvinylpyrolidone, ethyl cellulose, proteins, coprecipitates, ground mixtures, cyclodextrin complexes, suspensions, adsorbates, etc.

A number of difficulties, however, are encountered in the estimation of drug in the samples taken from such complex media. Dissolution rate measurement methods involve filtration of the dissolution medium samples through a conventional

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filter. The finer particles of drugs could pass through the filter medium and continue to dissolve in the filtrate. A soluble drug–excipient or drug–additive complex could also be present in the filtrate and be measured as free drug. In the latter case a specific assay must be utilized for the analysis of the filtrate.

Dialysis techniques have been adopted to overcome the problem of obtaining a representative sample of drug concentration from the dissolution fluid without substantially affecting the fluid volume (Marlowe and Shangraw, 1967; Barzilay and Hersey, 1968; Lovering and McRae, 1973; Shah and Sheth, 1976; Rosen and Macheras, 1984; Shrivastava et al., 1985). Nevertheless, these techniques suffer from one or more serious drawbacks. Most of them are adaptable only to the dissolution system described by the authors. The system of Rosen and Macheras (1984) differs significantly from those apparatuses recommended in the official compendia. The technique of Lovering and McRae (1973) is time-consuming since it requires the determination of the transfer rate of drug across the dialysis membrane under steady-state conditions. With the exception of the technique of Rosen and Macheras (1984) all the others exhibit various degrees of long equilibrium times between the drug concentration in the dissolution medium and that in the dialysis medium. Explicit relationships for the drug concentrations in the two compartments do not exist for most of the systems, and the evaluation of the dissolution characteristics is based on the drug concentration profiles obtained in the dialysis medium.

It is conceivable that a technique which is not time-consuming and can also be: (i) adaptable to the apparatuses recommended in the official compendia or those which have been reported in the literature; (ii) capable of providing the actual dissolution profiles; and (iii) flexible enough to be used on an automated or semi-automated basis, will undoubtedly be of value in all cases where dissolution samples require a specific separation step for the estimation of drug concentration.

Advances were made in this topic by the application of flow injection analysis (FIA) to the dissolution studies (Koupparis et al., 1984). Although powerful for monitoring drug dissolution from complex formulations this technique cannot be used when a dialysis treatment is unavoidable. However, the recently reported (Macheras et al., 1986) flow injection serial dynamic dialysis (FISDD) technique for protein binding studies has features which make its application to dissolution studies in complex media suitable.

The object of this work was to develop and evaluate the FISDD technique as a method which could be used to examine dissolution in complex media. Salicylamide, nitrofurantoin, acetaminophen and propantheline bromide were chosen as model drugs while low fat milk was used as a model dissolution medium. The drugs were chosen for this research on the basis of their diversity in physicochemical properties. The dissolution of drugs was studied in milk, because recent interest in drug–food interactions (Welling, 1984) make milk one of the most representative food simulating media for dissolution studies. Besides, the utilization of milk as an inert vehicle of drug delivery systems (Macheras and Reppas, 1986) is an additional reason for performing dissolution experiments in presence of milk. For comparative purposes the dissolution of drugs in buffer solutions was also studied.

Experimental

Reagents

All reagent solutions, referred to in Fig. 2, were prepared in de-ionized water from analytical grade materials. Propantheline bromide, salicylamide, nitrofurantoin, acetaminophen, and their formulations were obtained from commercial sources [1–4].

Drug standard solutions

Stock solutions of propantheline bromide (7500 μg/ml in water), salicylamide (1500 μg/ml in 0.01

[1] Propantheline bromide: Propanthine, Searle Ltd; Propanthelini, Livafarm.

[2] Salicylamide: Ilvico-Neo, Merck Hellas Ltd.

[3] Nitrofurantoin: Furadantin, Norwich Eaton; Furonitran, Defarm.

[4] Acetaminophen: Norgesic, Riker (Cana) S.A. Athens, Greece; Panadol, Sterling Drug Hellas (Winthrop).

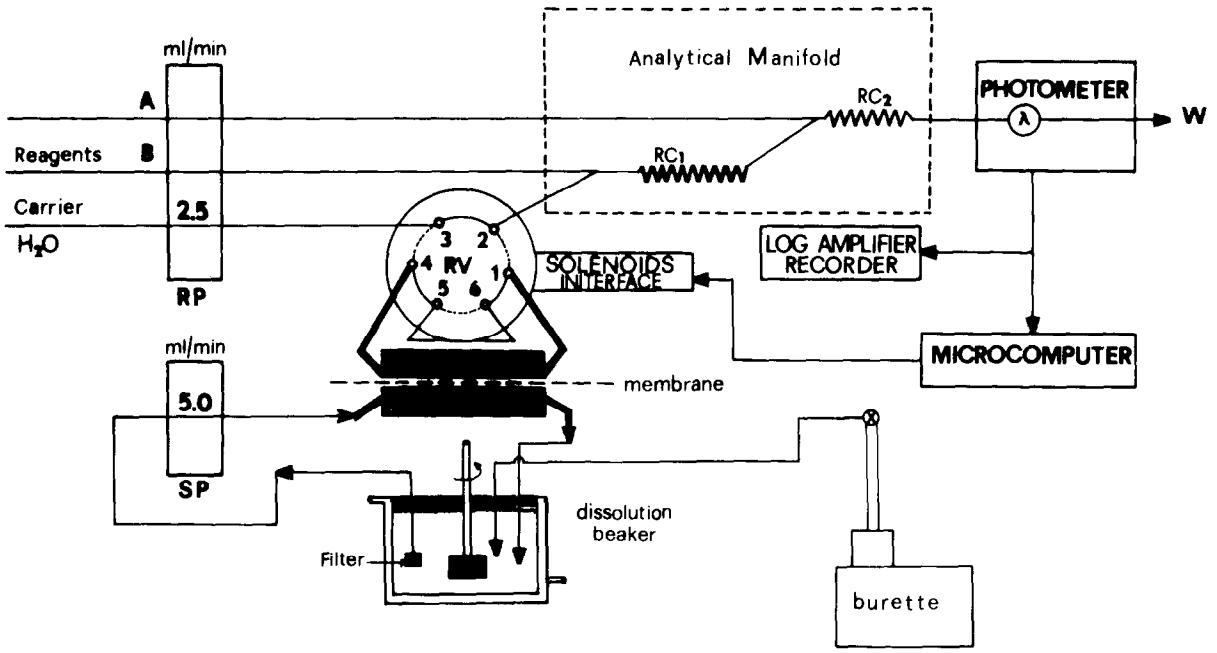

Fig. 1. Schematic diagram of flow injection serial dynamic dialysis (FISDD) system for dissolution studies in milk. RP, reagent pump; SP, sample pump; RC1, RC2, reaction coils; W, waste; RV, rotary valve.

M phosphate buffer pH 6.5 or milk), nitrofurantoin (4000 μg/ml in dimethylformamide) and acetaminophen (15,000 μg/ml in phosphate buffer or milk) were prepared by dissolving the appropriate weight to pure substance in the specified solvent. Working standard solutions in aqueous buffer or milk were prepared by appropriate dilution of the stock solutions. Standard solutions of drugs in milk were prepared fresh every day.

Phosphate buffer 0.010 M, pH 6.5

1.20 g of NaH_2PO_4 were dissolved in water, 16.3 ml of 0.10 M NaOH were added and diluted to 1 litre.

Milk

Low fat milk [5] (cow milk, 0.75% in fat) was used as dissolution medium.

Instrumentation

The FISDD system for dissolution studies in milk, shown in Fig. 1, was developed by interfacing a laboratory-constructed automated photometric flow injection analyzer (Koupparis and Anagnostopoulou, 1984) to a commercial dialyzer unit [6] of 670 mm^2 dialysis surface (Macheras et al., 1986) and a rotating basket apparatus. The basket was rotated at 60 rpm at 3 cm distance from the bottom of the beaker. Water was circulated from a bath through the double-wall beaker ensuring a constant temperature of 37 ± 0.5°C throughout the dissolution experiment. A cover of plexiglass was used over the beaker to keep the sample probe in a constant position. A semipermeable membrane [7] with 20 μm pores, hydrated for a short time before use was used in the dialyzer unit.

For the calibration of the FISDD system 'simulated dissolutions' were performed using an automatic multispeed burette [8] driven by a stepper motor. High concentration of the drug in suitable

[5] Landgenossenschaft Ennstal, Austria.

[6] Technicon Instruments Corporation, U.S.A.

[7] Cuprophan membranes Technicon Chem. Co.

[8] Sargent-Welch model S-11120-12, U.S.A.

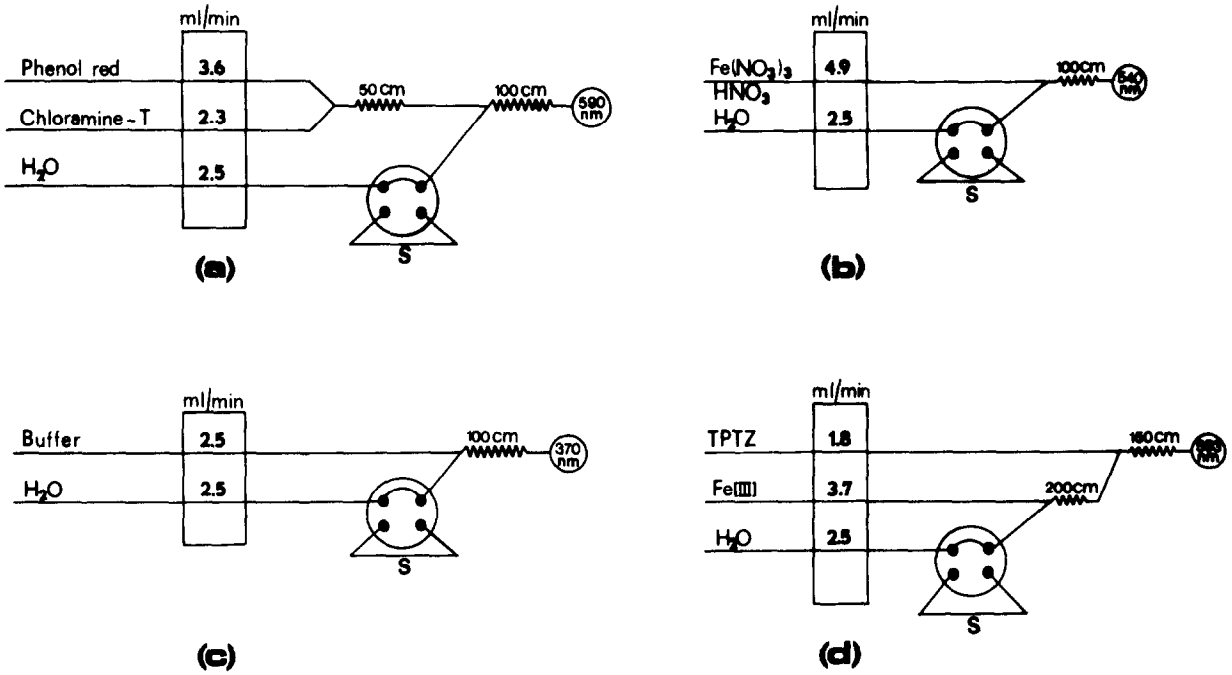

Fig. 2. Analytical manifolds for the FIA determination of: (a) propantheline bromide, phenol red 5.4×10^{-5} M in acetate buffer 1.0 M pH 4.6, Chloramine-T 3.5×10^{-3} M; (b) salicylamide, $Fe(NO_3)_3$ 0.05 M in HNO_3 0.03 M; (c) nitrofurantoin, phosphate buffer 0.01 M pH 6.5; (d) acetaminophen, TPTZ 1.0 mg/ml in 3.0×10^{-2} M HCl, Fe(III) 0.050 M in acetate buffer 0.30 M pH 3.6. S represents the receiving chamber of the dialyzer.

solvent was used to avoid extensive dilution of the dissolution medium. Water thermostated at 37°C was used as the carrier and receiving solution to avoid any contamination of the dissolution medium. The reagent pump can be automatically discontinued to avoid reagent consumption in prolonged dissolution experiments.

Analytical methods

The automated determination of the drugs was based upon well-known methods which were adapted to the FIA system. The analytical manifolds used for propantheline bromide, salicylamide, nitrofurantoin and acetaminophen, along with the reagents required, are shown in Fig. 2a, b, c and d, respectively. The automated colorimetric determination of bromide ions, based on their oxidation with chloramine-T and the subsequent bromination of phenol red at pH 4.6 to produce bromphenol blue monitored at 590 nm, was used to follow propantheline bromide (Anagnostopoulou and Koupparis, 1986). Salicylamide was determined using Trinder's reaction adapted to the FIA analyzer and monitored at 540 nm (Koupparis and Anagnostopoulou, 1986). Nitrofurantoin was monitored by direct measuring at 370 nm after mixing with phosphate buffer of pH 6.5. Acetaminophen was monitored using a recently developed FIA colorimetric method (Koupparis et al., 1985) based on its oxidation with iron (III) and subsequent chelation of the produced iron (II) with 2,4,6-tripyridyl-S-triazine (TPTZ).

Experimental procedure

To perform an automated dissolution experiment in milk using the proposed technique, the following general steps must be carried out.

(a) Construction and optimization of the analytical FIA manifold for the automated monitoring of the dialyzable drug in the receiving solution. The linear analytical range of the drug concentration, with the optimized mani-

fold and using a 30 s injection time, is then estimated by manual loading of the receiving chamber of the dialyzer with a series of standard drug solutions via the ports 5 and 6 of the rotary valve.

(b) The experimental dialytic rate constants of the tested drugs from the buffer and milk are then calculated as previously described (Macheras et al., 1986). This constant, in conjunction with the linear concentration range of the FIA determination from step (a) and the range of drug concentrations expected during the dissolution experiment are used to provide an optimum dialysis time.

(c) The calibration of the system is then carried out using the same volume of the buffer or milk and the other experimental parameters to be used in the dissolution experiment. An appropriate high drug concentration is used in the automatic burette to generate a 'simulated dissolution profile' with a concentration range and within a time analogous to the actual dissolution experiment. From the series of the peaks obtained (absorbance peaks vs time) a calibration curve can be constructed (absorbance peaks vs total drug concentration) using the known delivery rate of the burette. The time of injection of the dialyzate is used as the basis for the calculations. When milk is used as dissolution medium initial dialysis-measurement runs are carried out to determine any blank in the FIA determination associated with any dialyzable interfering substances from the milk.

(d) The actual dissolution experiment is then carried out as previously described (Koupparis et al., 1984). From the calibration curve obtained in step (c) in the same medium, the total drug concentration at the successive injection times is calculated and used to construct the dissolution profile of the drug and for further mathematical treatment.

Theory

The drug concentration in the dissolution medium changes continuously during dissolution and therefore its determination cannot be accomplished by the use of standard solutions as described in the protein binding studies with the FISDD system (Macheras et al., 1986). Instead, simulation experiments were carried out to mimic the actual dissolution process.

In the simulation experiments the rate of increase of drug concentration in buffer or milk is controlled by the delivery rate of the burette. Thus, the total drug concentration in buffer or milk, C_t, during the simulation experiment can be described by Eqn. 1,

$$C_t = U \cdot t \tag{1}$$

where U is the constant rate of concentration change by the burette, i.e. $U = dC_t/dt$, (calculated from the flow rate of the burette, the concentration of the standard drug solutions and the volume of the dissolution medium). Eqn. 1 shows a linear increase of the total drug concentration, C_t, in the dissolution medium.

The rate of dialysis of the drug dissolved in the dissolution medium is a function of its concentration, C_t. It would be expected that the permeation rate of drug would follow first-order diffusion kinetics,

$$\frac{dC_i}{dt} = K_{d_{b,m}}(C_t - C_i) = K_{d_{b,m}}(Ut - C_i) \tag{2}$$

where C_i is the instantaneous drug concentration in the receiving compartment and $K_{d_{b,m}}$ the apparent permeability rate constant of drug from either the buffer, K_{d_b}, or the milk solution, K_{d_m}.

A solution to this differential equation has the following form:

$$C_i = C_t\left(1 - \frac{1 - e^{-K_{d_{b,m}}t}}{K_{d_{b,m}} \cdot t}\right) \tag{3}$$

This equation describes the time profile of the dialyzate concentration in the receiving compartment during a dialysis run. Under given experimental conditions and dialysis time the term in the parenthesis of Eqn. 3 is constant for all successive dialysis runs. Therefore, the drug concentration in the receiving compartment at the termina-

tion of each dialysis run, $C_{i,t}$, is linearly related to the corresponding total drug concentration, $C_{t,t}$, in the dissolution medium. Generalizing for all successive dialysis runs, it can be written,

$$(C_{i,t})_n = L(C_{t,t})_n \quad (4)$$

where

$$L = 1 - \frac{1 - e^{-K_{d_{b,m}}t}}{K_{d_{b,m}} \cdot t} = \text{constant.}$$

Diagrammatically, Eqn. 1 is presented in Fig. 3a while Eqns. 3 and 4 are presented in Fig. 3b.

By using an appropriate FIA analyzer an analytical parameter is measured which is related to $C_{i,t}$ in a specific analytical range. As Eqn. 4 imposes the analytical parameter measured, absorbance peaks in the present study will be in turn directly related to $C_{t,t}$ in a specific range of concentrations. A calibration curve of absorbance peaks, A, vs total drug concentration in milk $C_{t,t}$ can be therefore constructed by performing simulation experiments,

$$A = a + bc_{i,t} = a + bLC_{t,t} = a + b'C_{t,t} \quad (5)$$

where a is the intercept and b′ is the slope of the regression line (Fig. 3c). By repeating the procedure with the test formulations the total drug concentration $C_{t,t}$ can be estimated from the calibration curve of Eqn. 5.

For the sake of completion of the theory the following points should be elucidated. The rate of dialysis of drug in dissolution–dialysis measurements depends upon its effective concentration in milk at any given time. The effective concentration is the drug concentration in solution available for dialysis that would be the concentration of free drug in solution (Macheras et al., 1986). This concentration was not known for the drug dissolution studies in milk while it was identical to the total drug concentration when the buffer was used. Therefore, the total concentration of drug in milk was used in Eqn. 2 in calculating apparent dialytic rate constants. Similar assumptions have been made by Shah and Sheth (1976) for their dissolution–dialysis system. This approximation can be also considered valid since the binding of drugs to milk proteins is expected to be low and concentration independent (Syversen and Ratkje, 1985; Rosen and Macheras, 1985). An additional concern arises from the difference in the type of drug concentration increase between simulation and actual dissolution experiments. In fact, the change of drug concentration is linear during the simulation experiments while concentration changes are mostly non-linear when actual dissolution takes place. However, the sampling in frequent time intervals, every 3 min or less in the present study, is obviously adequate for a valid approximation of a non-linear dissolution profile.

Finally, it should be noted that the use of a calibration curve calculated from the simulation experiments to estimate the total concentration of drug in milk, requires validation. According to the theory developed the same working calibration curve will result under various rates of drug concentration increase in the simulation experiments provided that all other experimental conditions are kept constant. Ideally, the range of drug concentration rate increase, encountered when actual dissolution is running, should be utilized to verify the applicability of the working calibration curve. The rationale for these studies is to ensure that the dialysis conditions chosen allow the dialysis rate measurements to reflect the dissolution behaviour. When unsuitable dialysis conditions are used or the dissolution rate is too fast, a build up of drug concentration in the milk will make the dialysis rate, rate limiting overall. Under these cir-

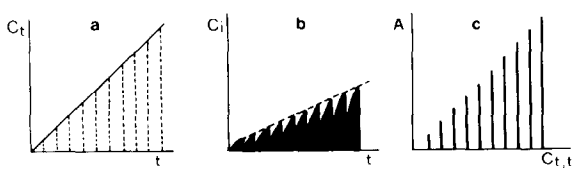

Fig. 3. Schematic depiction of the simulated dissolution experiment using FISDD system. a, linear change of drug concentration (C_t) in the dissolution medium according to Eqn. 1; b, change of drug concentration C_i in the receiving solution of the dialyzer unit of successive dialysis runs according to Eqn. 3. The concentrations $C_{i,t}$ correspond to the maxima of C_i peaks; c, recorded FIA absorbance peaks at successive injections according to Eqn. 5.

cumstances a linear calibration curve obtained should be used carefully.

Results and Discussion

The analytical characteristics of the 4 drug determinations using the FISDD system are shown in Table 1. As can be seen the values of the dialysis rate constant of the drugs in buffer are in inverse order to the size of the dialyzable species. In all cases the K_d values in milk were lower than those in buffer as the concentration of the free drug decreases because of a varying partition of the drugs in the milk phases and also of the decreased diffusion coefficient of the molecules in the milk matrix. With the dialysis time chosen for the 4 drugs, the percent dialysis from buffer was 76.4, 51.0 30.0 and 25.6% (Table 1). The corresponding values in milk were decreased at relative percentages of 18.1% for bromide ions, 13.7% for salicylamide, 10.0% for acetaminophen and 6.2% for nitrofurantoin. It seems that the order of the decrease in the percent of dialysis in milk follows the order of the decrease in the ionic and the hydrophilic character of the drugs. Similar decreases of the slope of the calibration curves of the drugs in milk occurred, the relative percentage decrease being 10.0, 14.3, 9.8 and 6.3% respectively.

The linearity of the calibration curves for both standard drug solutions in buffer and milk was excellent, with r ranging from 0.9993 to 0.99996.

A blank with excellent precision was found in the determination of salicylamide and acetaminophen using the FISDD system (Table 1). This was caused by dialyzable compound(s) from milk reacting with iron (III). The blank was subtracted from all salicylamide and acetaminophen measurements when milk was used as dissolution or dialysis medium.

As the determination of propantheline bromide

TABLE 1

ANALYTICAL CHARACTERISTICS OF THE FISDD DETERMINATIONS OF DRUGS IN PHOSPHATE BUFFER 0.01 M pH 6.5 AND MILK AT 37°C

Drug	Dialysis time (min)	Linear concentration range in dissolution medium (μg/ml)	Dialysis rate constant (K_d ($\pm$S.D.) min^{-1})	Percent [a] dialysis (%)	Equations of calibration curves [b]
Propantheline bromide	3.00	10–180	buffer: 0.481 $\pm$ 0.056 [c]	76.4 [c]	$A = -0.0395 + 0.006768 \cdot C$ $r = 0.9997$
			milk: 0.328 $\pm$ 0.035 [c]	62.6 [c]	$A = -0.0810 + 0.006091 \cdot C$ $r = 0.9998$
Salicylamide	2.50	10–600	buffer: 0.285 $\pm$ 0.033	51.0	$A = -0.0025 + 0.001566 \cdot C$ $r = 0.99993$
			milk: 0.231 $\pm$ 0.027	44.0	A [d] $= 0.0282 + 0.001342 \cdot C$ $r = 0.99996$
Acetaminophen	2.50	10–560	buffer: 0.143 $\pm$ 0.002	30.0	$A = 0.0014 + 0.001652 \cdot C$ $r = 0.99992$
			milk: 0.125 $\pm$ 0.002	27.0	A [d] $= 0.2588 + 0.001489 \cdot C$ $r = 0.9998$
Nitrofurantoin	3.00	10–150	buffer: 0.099 $\pm$ 0.01	25.6	$A = 0.0115 + 0.006004 \cdot C$ $r = 0.9997$
			milk: 0.091 $\pm$ 0.01	24.0	$A = 0.0175 + 0.005625 \cdot C$ $r = 0.9993$

[a] Calculated from the equation, $\% C_i = 100 (1 - e^{-K_d t})$ (Macheras et al., 1986).

[b] Obtained by measuring standard drug solutions in buffer and milk. Injection time 30 s.

[c] Actually of bromide ions.

[d] The constant blank is caused by dialyzable compound(s) from the milk.

was indirect and based on the counter ion bromide, an evaluation test for this determination was carried out. Samples of milk, containing 25–150 mg/l of propantheline bromide, were analyzed with FISDD system using a calibration curve obtained with standard bromide solutions in milk. The mean recovery was 100.6% (96.8–105.2%).

The precision of the FISDD system was tested in all cases by measuring standard drug solutions, both in buffer and milk in quadruple. The standard deviations ranged from 0.001 to 0.016 absorbance units and the relative standard deviation (RSD) was always less than 2%.

In order to evaluate the reliability of the FISDD technique to monitor dissolution, 3 'simulated dissolutions' with different rates of concentration change were carried out using propantheline bromide. The differences in the rate of concentration change were achieved using combinations of the speed of the burette and the concentration of the drug solution added. The results are shown in Table 2. As is shown the 3 calibration dissolution curves have a relative standard deviation of 0.5% and 30% for the slope and the intercept respectively. The linearity of these curves is excellent for such experiments with r varying from 0.998 to 0.9992. From these results it is clear that the new technique can be used to monitor dissolution where the rate of drug concentration increase varies considerably.

Summarized results from simulated dissolutions in buffer and milk for the 4 drugs are shown in Table 3. As is shown, the linearity of these simulated dissolution curves is good for such type of experiments ($r = 0.995$–0.9998) showing the validity of Eqn. 5 with sufficient precision of the parameters of the curve equations (intercept and slope) for various rates of concentration change. The slopes of the curves for buffer were always statistically higher (confidence level 95%) than those obtained for milk. By subtracting the slopes of the calibration curves in the two media, the relative magnitude of the reductions were found to be, −7.1% for propantheline bromide (actually bromide), −13.5% (mean) for salicylamide, −14.8% for acetaminophen and −9.4% for nitrofurantoin. These decreases (dynamic conditions) are of the same level as the decreases in the calibration curves of drugs (static conditions) in buffer and milk discussed above. It is obvious that simulated dissolution experiments for each drug in milk are required for calibration of the system.

TABLE 2

SIMULATED DISSOLUTIONS OF PROPANTHELINE BROMIDE IN PHOSPHATE BUFFER (100 ml) 0.01 M pH 6.5

Drug concentration [a] (μg/ml)	Drug delivery rate [a] ($\mu g \cdot ml^{-1} \cdot min^{-1}$)		
	I	II	III
	4.905	4.905	9.810
	Absorbance peaks		
29.4	0.043	0.050	0.005
44.1	0.093	0.108	–
58.9	0.150	0.175	0.105
73.6	0.225	0.243	–
88.3	0.295	0.325	0.238
103.0	0.365	0.405	–
117.7	0.410	0.462	0.383
132.4	0.500	0.523	–
147.2	0.565	0.559	0.525
161.9	0.625	0.625	–
176.6	0.691	0.733	0.660

Calibration dissolution curves

I: $A = (-0.103 \pm 0.006) + (0.004495 \pm 0.000058) \cdot C_{t,t}$
$r = 0.9992$

II: $A = (-0.084 \pm 0.012) + (0.004528 \pm 0.000106) \cdot C_{t,t}$
$r = 0.998$

III: $A = (-0.149 \pm 0.014) + (0.004542 \pm 0.000120) \cdot C_{t,t}$
$r = 0.9990$

I = burette speed, 0.06533 $ml \cdot min^{-1}$; drug concentration, 7500 $\mu g \cdot ml^{-1}$. II = burette speed, 0.13066 $ml \cdot min^{-1}$; drug concentration 3750 $\mu g \cdot ml^{-1}$. III = burette speed, 0.13066 $ml \cdot min^{-1}$; drug concentration 7500 $\mu g \cdot ml^{-1}$.

[a] Calculated from the speed of burette, the concentration of the drug solution and the volume of the dissolution medium.

Usually a negative intercept was found in the simulated dissolution experiments (except for the cases showing a determination blank, as for salicylamide and acetaminophen) because of a precise delay time in the whole procedure (mixing of the drug with the rather slow rotating basket, dialysis under dynamic conditions, FIA measurement).

Figs. 4–7 show some typical dissolution profiles obtained for various commercial tablets of the drugs studied with the proposed technique. The dissolution profiles of propantheline bromide

TABLE 3

EQUATIONS OF SIMULATED DISSOLUTION EXPERIMENTS OF THE DRUGS IN BUFFER AND MILK

Buffer	Milk
Propantheline bromide (dissolution medium: 250 ml)	
$A^a = -0.131\ (\pm 0.004) + 0.00648\ (\pm 0.00004) \cdot C_{t,t}$ $r = 0.9998$	$A^a = -0.143\ (\pm 0.008) + 0.00602\ (\pm 0.00008) \cdot C_{t,t}$ $r = 0.9991$
	$A^b = -0.203\ (\pm 0.009) + 0.00593\ (\pm 0.00008) \cdot C_{t,t}$ $r = 0.9996$
Salicylamide (dissolution medium : 100 ml)	
$A^c = -0.005\ (\pm 0.001) + 0.00135\ (\pm 0.00002) \cdot C_{t,t}$ $r = 0.995$	$A^c = 0.019\ (\pm 0.001) + 0.00124\ (\pm 0.00002) \cdot C_{t,t}$ $r = 0.998$
$A^d = -0.025\ (\pm 0.004) + 0.00146\ (\pm 0.00004) \cdot C_{t,t}$ $r = 0.998$	$A^d = 0.015\ (\pm 0.002) + 0.00119\ (\pm 0.00002) \cdot C_{t,t}$ $r = 0.999$
Nitrofurantoin (dissolution medium : 500 ml)	
$A^e = -0.0229\ (\pm 0.0008) + 0.002232\ (\pm 0.000008) \cdot C_{t,t}$ $r = 0.9998$	$A^e = -0.0223\ (\pm 0.0006) + 0.002021\ (\pm 0.000007) \cdot C_{t,t}$ $r = 0.9998$
Acetaminophen (dissolution medium : 900 ml)	
$A^f = -0.003\ (\pm 0.003) + 0.00169\ (\pm 0.00003) \cdot C_{t,t}$ $r = 0.9992$	$A^f = 0.017\ (\pm 0.003) + 0.00144\ (\pm 0.00003) \cdot C_{t,t}$ $r = 0.999$
	$A^g = 0.033\ (\pm 0.006) + 0.00148\ (\pm 0.00006) \cdot C_{t,t}$ $r = 0.998$

Rate of concentration change ($\mu g \cdot ml^{-1} \cdot min^{-1}$): a, 3.924; b, 7.848; c, 1.962; d, 4.905; e, 5.232, f, 5.450; g, 10.90.

and salicylamide in milk were remarkably lower than the corresponding ones obtained in buffer (Figs. 4 and 5). The inappreciable dissolution of salicylamide from the coated tablet in milk would be probably due to the limited wetting of the formulation which retards the disintegration of the formulation. The differences in the dissolution rates of propantheline bromide observed in the two media, might be linked with the hydrophilic character of the drug molecules. Similar dissolution profiles were obtained with the nitrofurantoin formulation in both media (Fig. 6). Nevertheless, the dissolution curve in buffer reaches a plateau probably due to solubility limitations while the concentration in milk is progressively rising. A plausible explanation could be a continuing dissolution–partition of nitrofurantoin in the milk phases resulting in an overall increase of nitrofurantoin solubility in milk. The dissolution profiles of an acetaminophen tablet indicate again

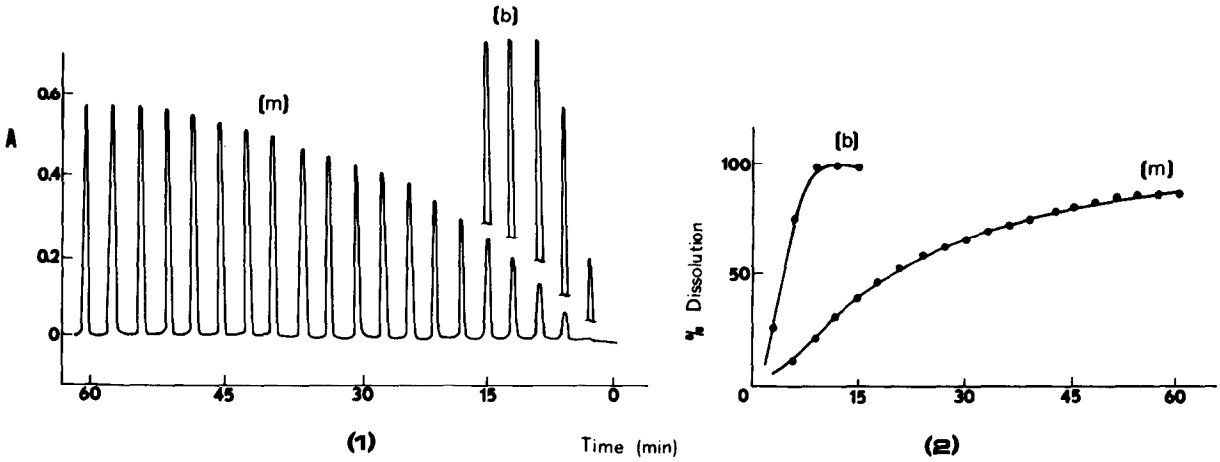

Fig. 4. Dissolution profiles of a propantheline bromide tablet (15 mg, Propanthine, Searle Ltd.), in 250 ml of phosphate buffer (b) and milk (m). From the FIA absorbance peaks recorded (1) the % dissolution was calculated using the corresponding 'simulated dissolution' curve (2). Sampling every 3 min.

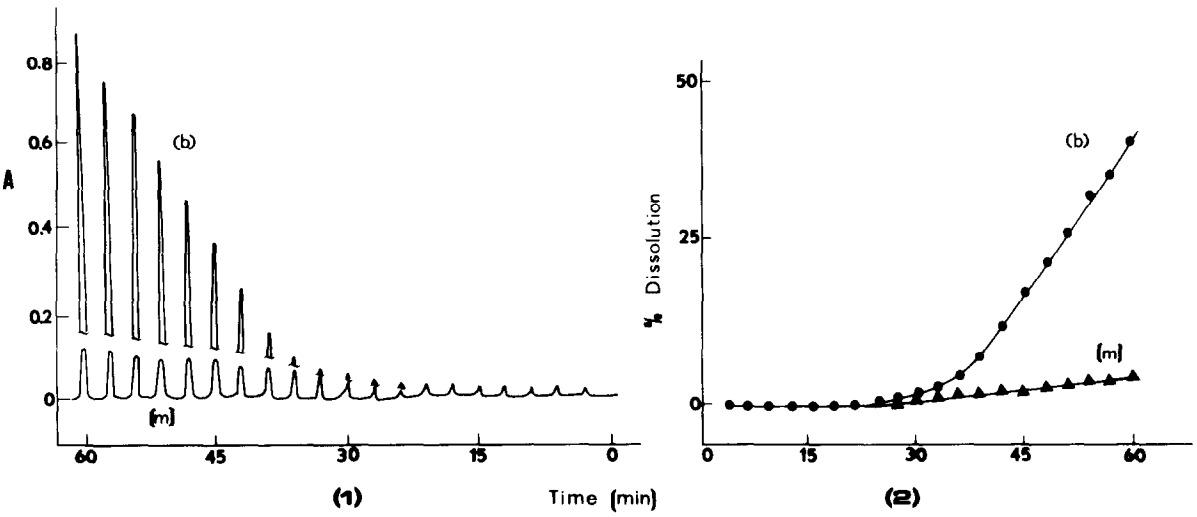

Fig. 5. Dissolution profiles of a salicylamide sugar coated tablet (150 mg, Ilvico-Neo, Merck), in 100 ml of phosphate buffer (b) and milk (m). From the FIA absorbance peaks recorded (1) the % dissolution was calculated using the corresponding 'simulated dissolution' curve (2). Sampling every 3 min.

a decreased dissolution rate of the drug in milk (Fig. 7).

For comparative purposes, the values of the dissolution rate constants for all drugs and formulations examined were calculated by linear regression analysis based on the logarithmically transformed integrated form of the Noyes–Whitney equation,

$$\ln\left(1 - \frac{C_t}{(Q/V)}\right) = -K(t - t_o) \qquad (6)$$

where Q is the quantity of drug in the formulation, V is the volume of the dissolution medium, K is the apparent dissolution rate constant and t_o the lag-time observed experimentally. The values of K obtained and the correlation coefficients of equations are shown in Table 4. In all cases an acceptable linearity ($r = 0.95$–0.995) was noted. The shape of the dissolution curves (Figs. 4–7) and the results quoted in Table 4 allow some conclusions to be reached. The degree of the reduction of the dissolution rate of drugs in milk varies according to the chemical structure of the drug and the formulation factors. Overall, the presence of milk is likely to affect the dissolution process of the examined drugs in the following ways: (i) retard almost completely the dissolution of salicylamide from the coated formulation; (ii) reduce dramatically the dissolution rate of the hydrophilic propantheline bromide; (iii) reduce to a lesser extent the dissolution rates of more hydrophobic nitrofurantoin and acetaminophen. It is interesting to note that the saturation solubility of

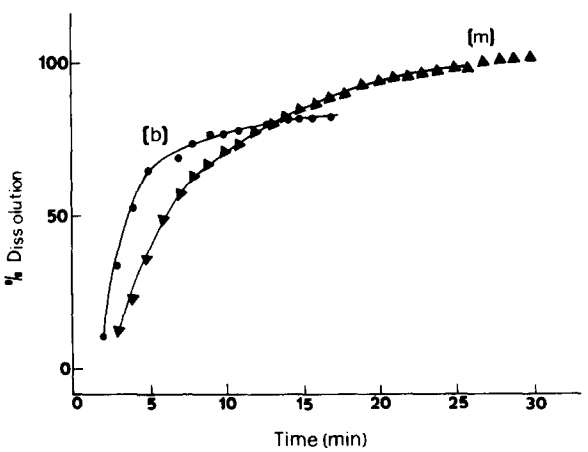

Fig. 6. Dissolution profiles of a nitrofurantoin tablet (100 mg, *Furonitran*, Defarm), in 500 ml of phosphate buffer (b) and milk (m). Sampling every 1 min. For the sake of clarity FIA absorbance peaks are not shown.

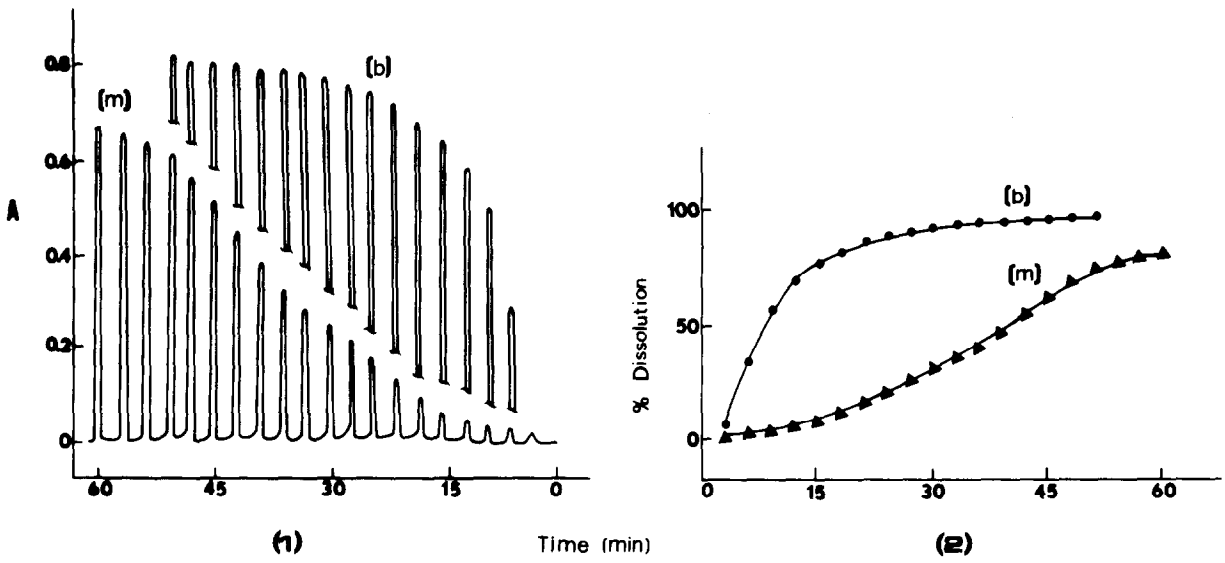

Fig. 7. Dissolution profiles of an acetaminophen tablet (500 mg, *Panadol*, Sterling Drug Hellas S.A.), in 900 ml of phosphate buffer (b) and milk (m). From the FIA absorbance peaks recorded (1) the % dissolution was calculated using the corresponding 'simulated dissolution' curve (2). Sampling every 3 min.

propantheline bromide and acetaminophen did not alter in presence of milk while nitrofurantoin solubility seems to be enhanced in milk. The latter observation might be linked with the well-known fact that the bioavailability of nitrofurantoin is enhanced when administered with food (Welling, 1984).

The validity of the FISDD technique for monitoring dissolution studies in complex media was demonstrated. The use of this system eliminates the need for: (i) a specific assay of drug; (ii) extraction procedures; and (iii) recovery studies. In addition to these, the interface of the FISDD system with the dissolution apparatuses furnishes

TABLE 4

DISSOLUTION RATE CONSTANTS OF DRUG FORMULATIONS IN PHOSPHATE BUFFER AND MILK

Drug (Formulation)	Dissolution rate constant [a] (min^{-1}) – correlation coefficient of Eqn. 6	
	Buffer	Milk
Propantheline bromide		
(Propanthine)	0.080 (±0.026) $-r = 0.87$	0.017 (±0.001) $-r = 0.98$
(Propanthelini)	0.035 (±0.002) $-r = 0.97$	0.0082 (±0.0005) $-r = 0.98$
Salicylamide		
(Ilvico-Neo)	0.016 (±0.001) $-r = 0.98$	0.00115(±0.00007) $-r = 0.98$
Nitrofurantoin		
(Furadantin)	0.0060(±0.0002) $-r = 0.991$	0.0036 (±0.0002) $-r = 0.97$
(Furonitran)	0.161 (±0.019) $-r = 0.96$	0.145 (±0.005) $-r = 0.995$
Acetaminophen		
(Norgesic)	0.032 (±0.001) $-r = 0.994$	0.0029 (±0.0001) $-r = 0.993$
(Panadol)	0.071 (±0.005) $-r = 0.98$	0.032 (±0.003) $-r = 0.95$

[a] Standard deviation in parentheses.

all the features which are related to the automation of the technique as described elsewhere (Koupparis et al., 1984). The technique can be useful even in the case when an automated FIA method is not available for on-line monitoring of the dialyzable drug. In this case the successive dialyzates are collected in tubes and analyzed manually by acceptable analytical procedures.

Due to the recent interest in food–drug and food–formulations interactions, it can be anticipated that food simulating media will be progressively used more frequently. Although bioavailability studies are for the time being conducted with fasting subjects, the continuous increase in sustained release formulations will necessitate their in vitro evaluation under conditions which are more akin to the in vivo situation. In this case the value of the proposed system in evaluating in vitro dissolution in food simulating media is self-evident. It is hoped that such experiments will provide in vitro data which give better than rank order correlations with the in vivo results.

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