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A Method to study the Kinetics of Oral Mucosal Drug Absorption from Solutions

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Abstract—A method has been developed which allows the kinetics of oral mucosal drug absorption from solutions to be studied in a single 15–20 min test. This is an improvement over the traditional buccal absorption test which requires a separate experiment for each time point, thus taking days for each kinetic profile. It should also allow for disappearance of drug from the mouth and appearance in the plasma to be monitored simultaneously. The drug solution includes 8 $\mu g\ mL^{-1}$ phenol red as a marker for salivary secretion. Samples are withdrawn at suitable times with a positive displacement pipettor and analysed for drug and phenol red. Salivary volume time data are described by least squares polynominals. Kinetic parameters are estimated by non-linear least squares regression. The method, which requires that phenol red is not lost during a test, provides an internal test for this. It also requires that phenol red and the drug do not interfere with each other's assay, that saliva does not affect the assays of these materials, and that phenol red does not interfere with the absorption of the drug.

There is a growing interest in the potential use of the oral mucosae for drug delivery. This route offers advantages for drugs which undergo high first-pass metabolism or are unstable in the gastrointestinal tract. Since the mouth has the most readily accessible mucosal membranes it is a potential platform for controlled release devices. The rational design of these delivery systems requires, among other things, information on the kinetics of absorption from the mouth.

The buccal absorption test was introduced by Beckett & Triggs (1967) as an in-vivo model of kidney tubule absorption. It has been used subsequently to study: oral mucosal absorption, pH effects, drug structure absorption relationships (Beckett & Hossie 1971) and drug excipient interactions (McElnay et al 1982; McElnay & Temple 1982). Basically, the method is to swirl a buffered drug solution in the mouth for a given time after which it is expelled, the mouth rinsed and the combined solution and rinse analysed for remaining drug. The mass absorbed is determined by difference between mass swirled and mass recovered. Kinetic information is obtained by repeating this procedure for differing times usually up to a maximum of 15 min. A suitable wash-out period between swirlings has not been reported. If 2 h is assumed, then 2 days is required to obtain sufficient data for one kinetic profile. Since pH often changes due to salivary secretions an average of starting and final pH values is used in computations.

Two modifications to this buccal absorption test have been proposed. Firstly, Dearden & Tomlinson (1971) pointed out that salivary secretion alters the kinetics by changing the concentration of drug in the mouth. They proposed a linear relationship between salivary secretion and time, thus:

$$\frac{-dm}{dt} = \frac{k.C}{V_i + vt} \qquad (1)$$

where m and C are the mass and concentration of drug in the mouth at time t, V_i the volume of solution put into the mouth initially and v salivary secretion rate.

Secondly, Schurmann & Turner (1978) included a non-absorbable marker (phenol red) within the drug solution to monitor for accidental swallowing of the solution.

This paper reports on a modified method using verapamil as a model drug. In this method multiple samples are withdrawn from the mouth enabling the kinetic data to be collected in a single trial. Phenol red is included as a marker enabling salivary secretion to be compensated for, and it is also used to monitor for accidental swallowing. pH can also be continually monitored allowing changes in it to be incorporated into the numerical analysis. The method should also enable drug disappearance from the mouth and appearance in the plasma to be determined simultaneously. Preliminary results on this method have been presented previously (Tucker 1987).

Materials and Methods

Materials

The following were used: verapamil hydrochloride BP and norverapamil (kindly supplied by Reckitt & Colman, Australia), propranolol HCl (kindly supplied by ICI, Australia), phenol red (Difco Laboratories, Surrey, UK), acetonitrile-190 (Waters Associates, Australia), triethylamine (Sigma, USA), borax BP (David Craig, Bisbane, Australia), sodium chloride AR, disodium hydrogen phosphate AR and sodium hydroxide AR (Ajax Chemicals, Australia), boric acid AR, sodium dihydrogen phosphate AR and orthophosphoric acid AR (BDH, Australia).

A stock solution of verapamil HCl (1% in glass distilled water) was stored under refrigeration and used as required. A phenol red solution (ca 0·008% in glass distilled water) was prepared by stirring 80 mg of phenol red in 1L of distilled water for 2 h, then filtering (0·22 μm Millipore GVWP 047 00) to remove undissolved solids. This solution was stored in the dark.

Modified buccal absorption test

The method has been developed using verapamil concentrations of 1, 2, 4, and 8 mg/25 mL at pH 7·9 and 5mg/25 mL at

pH values of 6·5, 7·0, 7·5, 8·0, 8·5. Tests were performed in duplicate in two trained volunteers separated by a minimum period of 5 h.

Solutions for swirling in the mouth were prepared in isotonic borate and phosphate buffers (Anderson 1983) by adding the appropriate volume of verapamil HCl stock solution (e.g. 2 mL/100 mL for a 5 mg/25 mL solution) and 10 mL/100 mL of phenol red stock solution. The final phenol red concentration was about 8 μg mL.$^{-1}$

Before each buccal test, the trained volunteer moistened his/her mouth with a few mL of distilled water.

For each buccal test a 1 mL aliquot of the swirling solution was taken for analysis then about 25 mL of the solution was weighed into a tared beaker. This was then taken into the mouth and swirled about 60 times a minute by movement of the cheeks and tongue and swallowing avoided. The beaker was reweighed to get the exact weight and hence volume of the sample swirled.

Typically, samples were taken at 0·1, 1, 2, 3, 4, 6, 8, 10, 12, and 15 min with a Brand (W-Germany) 1 mL macro-Transferpettor. To achieve this without any loss, the volunteer tilted his head slightly backwards then slipped the plastic pipette tip between tightly closed lips. The head was then inclined so a sample could be withdrawn without entrapped air. Samples were analysed for verapamil (HPLC), phenol red (visible spectroscopy) and pH (Metrohm 9100 AG Herisau microelectrode). After the final sample the remaining solution (ca 30 mL) was expelled into a 50 mL volumetric flask and the mouth rinsed for 10 s with ca 10 mL of distilled water. The combined solution and wash was adjusted to volume after most of the froth had collapsed. This was also analysed for verapamil and phenol red.

Sample preparation

After measurement of the pH, a 0·9 mL portion was taken and 0·1 mL of internal standard (propranolol HCl 1 mg mL^{-1}) and 1 mL of acetonitrile added. The solution was vortexed then centrifuged at 2000 rev min^{-1} for 10 min (Hettich Universal). A 0·5 mL sample of supernatant was taken for analysis by HPLC. 75 μL of 2·5 M sodium hydroxide was added to the remaining solution which was revortexed and again centrifuged. The supernatant was analysed at 563 nm for phenol red.

The small amount of protein present was precipitated by the acetonitrile. The concentration of sodium hydroxide was sufficient to raise the pH to about 12. If too much was added, the acetonitrile was salted out producing a two phase system which could not be analysed for phenol red.

Phenol red analysis – Beer Lambert calibration

Standard solutions were prepared in duplicate in isotonic borate buffer. Each standard was mixed with an equal volume of acetonitrile, alkalinized, vortexed and centrifuged as above, then analysed at 563 nm (Varian SuperScan 3).

The effect of saliva on the calibration was determined by repeating this procedure using isotonic borate buffer which had been swirled in the mouth for 5 min by the trained volunteers.

The effect of verapamil and propranolol on the phenol red assay was determined by preparing triplicate solution containing 8 μg mL^{-1} phenol red with and without verapamil HCl (5 mg/25 mL) and propranolol HCl (0·1 mg mL^{-1}).

HPLC analysis of verapamil

The following were used: mobile phase (acetonitrile 39% w/w, triethylamine 0·1% v/w, sodium dihydrogen phosphate dihydrate 0·435% w/w, phosphoric acid or sodium hydroxide sufficient to adjust the pH to 7·4, and water up to 100% w/w); flow rate 1 mL min^{-1}; column: Waters Nova-Pak C18 with a Rheodyne 2 μm frit prefilter; detector: Waters 450 variable wavelength detector at 278 nm; pump: Waters 6000A; injector: 20 μL injection with a Waters U6K injector or a Kortec K65B automated sample injector; Waters Data Module for integration of the peak areas.

A calibration was carried out by preparing duplicate standard solutions of verapamil HCl in buffer and buffer-saliva. The final concentrations of verapamil HCl were 0·05, 0·1, 0·15 and 0·2 mg mL^{-1}. These were assayed as described above.

Effect of phenol red on verapamil absorption

The method requires that the phenol red does not interfere with the absorption of the verapamil. To test this, verapamil alone (5 mg/25 mL) and verapamil (5 mg/25 mL) plus phenol red (8 μg mL^{-1}) solutions, both at pH 7·9, were used in the Beckett & Triggs (1967) buccal absorption test using 5 min swirling. Tests were carried out in quadruplicate, morning and afternoon separated by at least 5 h over four consecutive days, in the two volunteers. The verapamil absorbed was determined by the difference between the mass swirled and that recovered.

Results and Discussion

Analyses

Since the salivary content of the samples increases from first to last, it was necessary to ensure that the analysis of the phenol red was not affected by saliva. Regression analysis (Draper & Smith 1968) of the calibration data for phenol red in borate buffer and buffer-saliva demonstrated that the data were adequately described by linear equations with zero intercepts. Slopes, plus/minus standard deviations of $0{\cdot}5461 \pm 0{\cdot}0016$ in buffer and $0{\cdot}5420 \pm 0{\cdot}0026$ in buffer-saliva were not significantly different ($P > 0{\cdot}25$) indicating that the saliva did not affect the phenol red assay.

The mean absorbance of triplicate solutions of phenol red alone and with verapamil and propranolol were not significantly different ($P > 0{\cdot}25$) indicating that these also did not interfere with the assay of phenol red.

The HPLC chromatograms had near-symmetrical, baseline resolved peaks with typical retention times of: propranolol 2·1–2·3, norverapamil 3·6–4·0, and verapamil 5·0–6·0 min. Phenol red eluted at the solvent front and had no effect on the peak areas of verapamil and propranolol. Peak area ratios versus concentration of verapamil in water and buffer-saliva were adequately described by linear equations with zero intercepts. The slopes were not significantly different ($P > 0{\cdot}25$) indicating that saliva did not affect the calibration. The sensitivity of the assay was 5 μg mL^{-1} with a within-day coefficient of variation of 2% in water and 5·4% in pooled saliva. The larger variation in the latter case probably reflects

the greater variability in measurement of proteinaceous solutions.

Effect of phenol red on absorption

Analysis of variance found no significant difference ($P > 0.25$) between verapamil alone and verapamil with phenol red. Thus phenol red did not affect the absorption of verapamil. The analysis also found no difference between morning and afternoon tests indicating that 5 h was a sufficient wash out period.

Modified buccal absorption test

Results of a typical buccal absorption test are shown in Table 1.

Saliva volumes. Saliva volume secreted Vs(j) in the intersampling period between the j-1 and j^{th} samples was calculated from the change in the phenol red absorbance at 563 nm using the following formulae:

$$V(j) = A(j\text{-}1).\ V(j\text{-}1)_a/A(j) \quad (2)$$

$$Vs(j) = V(j)\text{-}V(j\text{-}1)_a \quad (3)$$

where V(j) is the volume (solution plus saliva) in the mouth just before the j^{th} sample, $V(j\text{-}1)_a$ is the volume in the mouth just after the $(j\text{-}1)^{th}$ sample, A(j-1) and A(j) are the absorbances of the j-1 and j^{th} samples, respectively. A(0) is the absorbance of the initial swirling solution, that is time zero in Table 1. Also:

$$V(j\text{-}1)_a = V(j\text{-}1)\text{-}V_{sample} \quad (4)$$

where the volume of sample used in this trial was 1 mL. In initial tests a small negative value (up to −1·8 mL) was obtained for Vs(1). This could be due to random errors or to absorption of water from the swirling solution through the mucosae. In subsequent trials the volunteers moistened their mouths with distilled water. This seemed to overcome the problem.

The cumulative volume of saliva secreted up to the j^{th} sample was calculated by summing Vs(i) for i = i to j. Typical saliva secretion curves are shown in Fig. 1. Least squares polynomials were used to describe this cumulative data. The fits of the polynomials were judged by the runs test (Draper & Smith 1968) for non-randomness of residuals. In most cases quadratic polynomials were required, in a few cases linear equations were adequate, while in two cases cubic polynomials were necessary.

The above method for determining saliva volumes assumes that phenol red is not lost in any way. Losses could occur by swallowing, absorption, and adsorption to mucosal surfaces. However, the method provides an internal check for losses. The mass of phenol red put into the mouth was known from the concentration and volume of swirling solution. The mass recovered was calculated by summing the masses of phenol red in the 1 mL samples and that in the expelled solution. Over 35 trials the average loss was 3·1% with a range of −12 to +13%.

Verapamil absorbed. The mass of verapamil absorbed (or lost from the swirling solution) during an intersampling period was calculated from the concentration (C) of verapamil and the volume of solution in the mouth at the start and finish of the period. That is, the mass absorbed between the j-1 and j^{th} samples (M(j)) is:

$$M(j) = C(j-1).\ V(j-1)_a.\ -C(j).\ V(j) \quad (5)$$

The cumulative mass absorbed up to the j^{th} sample was obtained by summing M(i) for i = 1 to j. Typical results are shown is Table 1 and Fig. 2. The verapamil disappeared rapidly from the swirling solution and reached an apparent equilibrium in 5–10 min. Kates (1977), using the Beckett & Triggs (1967) buccal absorption test, demonstrated a similar behaviour for propranolol. Some curves showed a slight fall at 15 min. This could be due to the small drop in pH causing verapamil to partition back out of the membrane.

The mass of verapamil absorbed by the end of the trial period was known from the cumulative mass absorbed up to

Table 1. A typical data set for the buccal absorption of verapamil showing the sample times and pH's, the phenol red absorbance at 563 nm, the volume of saliva secreted in each interval, the cumulative (Σ) saliva volume, the verapamil absorbed in each interval (abs), and the cumulative (Σ) mass or percentage absorbed. The swirling solution contained verapamil (4·3 mg/25 mL) and phenol red (8 $\mu g\ mL^{-1}$) at pH 7·9.

					Verapamil			
Time (min)	pH	Absorb at 563 nm	Saliva (mL)	Σ(Saliva) (mL)	Concn (mg/25 mL)	Abs (mg)	Σ(Abs) (mg)	Σ(Abs) (%)
0	7·9	0·430	0	0	4·29	0	0	0
0·1	7·84	0·428	0·12	0·12	4·22	0·04	0·04	0·9
1	7·82	0·402	1·54	1·66	3·36	0·61	0·65	15·4
2	7·81	0·393	0·56	2·22	2·80	0·48	1·13	26·7
3	7·79	0·386	0·43	2·65	2·48	0·26	1·39	32·8
4	7·78	0·376	0·62	3·27	2·19	0·21	1·60	37·8
6	7·75	0·360	1·02	4·29	1·79	0·29	1·89	44·6
8	7·71	0·343	1·14	5·43	1·58	0·12	2·01	47·5
10	7·66	0·325	1·28	6·71	1·42	0·07	2·08	49·1
12	7·59	0·305	1·53	8·24	1·25	0·08	2·16	51·0
15	7·54	0·284	1·77	10·01	1·15	0·01	2·17	51·3

Verapamil absorbed calculated from the expelled solution concentration was 2·29 mg or 54·1% (cf 51·3% above). The percentage of phenol red lost, was 8·5%. The percentage of verapamil accounted for in samples, expelled solution and absorbed was 97·1%.

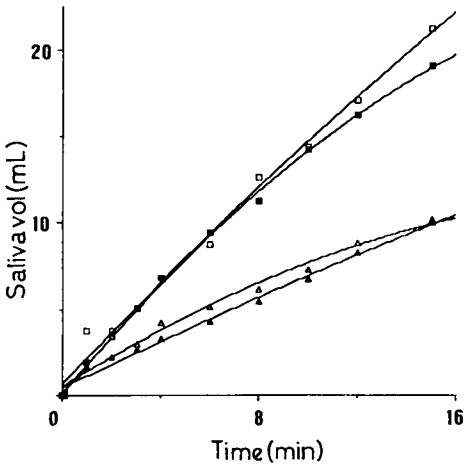

FIG. 1. Saliva secreted during some typical absorption tests. The saliva volumes were calculated from the change in the spectrophotometric absorbance (563 nm) of phenol red. The lines are the best fit quadratic polynomials to data for subjects IT (□ ■) and SH (△ ▲).

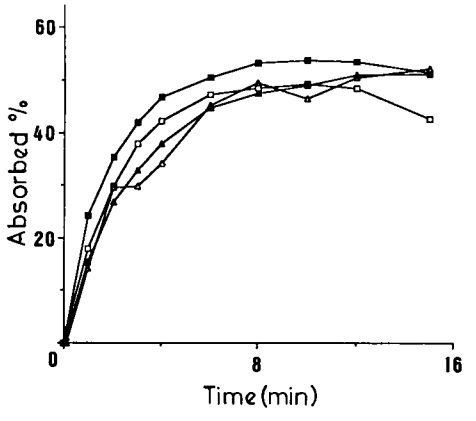

FIG. 2. The cumulative percentages of verapamil absorbed during the absorption tests of Fig. 1. Subjects: IT (□ ■), SH (△ ▲).

the last sample, but was also calculated from the mass recovered in the expelled solution (Table 1). These two methods gave results which agreed reasonably well and provided an internal check of the assays and calculations. A further check was made by calculating the percentage of the dose accounted for by summing the mass of verapamil in each of the 1 mL samples, with that in the expelled solution and cumulative mass absorbed.

pH. Changes in the pH are shown in Table 1. Differences in the starting and finishing pH's of solutions used in the original buccal absorption test have been observed previously. These have either been neglected, averaged (Schurmann & Turner 1978), or compensated for in an analogue computer fit of the data (Beckett & Moffat 1970).

Kinetic analysis. The verapamil concentration data were treated by non-linear regression to estimate the appropriate kinetic parameters. Given that $C = m/V$ and m and V are both varying with time, differentiation gives:

$$dC/dt = (dm/dt)/V - (dV/dt).C/V \quad (6)$$

where m and C are the mass and concentration of verapamil in the swirling solution, and V is the volume of swirling solution at time t. This volume equals:

$$V = V_i + V_{saliva} - V_{samples} \quad (7)$$

where V_i is the volume put into the mouth originally, V_{saliva} is the volume of saliva secreted up to time t and it was described by an empirical polynomial, and $V_{samples}$ is the total volume of the samples taken up to time t. The derivative dV/dt in the intersampling period was obtained by differentiation of the empirical polynomial. Samples were assumed to be withdrawn instantaneously.

Various models were considered for dm/dt. A simple first order model was found to be inadequate by the runs test for most data sets. This was expected given the apparent equilibrium nature of the data (Fig. 1). An appealing alternative was:

$$\text{Swirling solution} \rightleftharpoons \text{membrane} \rightarrow \text{blood} \quad (8)$$

however, in all cases the data were adequately described by the simpler equilibrium model:

$$\text{Swirling solution} \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} \text{membrane} \quad (9)$$

$$\text{Thus } dm/dt = -k_{12}.f.C + k_{21}.C_2 \quad (10)$$

$$= -k_{12}.f.C - k'_{21}.(m_i - m) \quad (11)$$

where C_2 is the concentration in kinetic compartment 2 (i.e. membrane in 9) m_i is the initial mass put into the mouth, k'_{21} equals k_{21} divided by the volume of kinetic compartment 2, and f is the fraction of verapamil unionized. Attempts to use the more complex model (8) were unsuccessful because of non-convergence of the program or resultant nonsensical estimates with enormous confidence limits. This was expected given the adequacy of the simpler model (9). If this kinetic model reflects the physiological description in (9), it suggests that the verapamil accumulates in the membrane from which it is perhaps lost only very slowly into the blood. This accumulation has been reported previously by Davis & Johnston (1979).

Parameters estimated in the fitting procedure were k'_{12} ($= k_{12}.f$), k'_{21}, and m_i. Thus f was assumed to be constant during each test period but this was not strictly true given the observed shift in pH. A more complex treatment might take this shift into account by deriving an empirical relationship between pH and time. However, the situation is more complex than this, since only bulk pH's were measured and there is evidence for a surface pH differing from the bulk pH (Schurmann & Turner 1978). It is the f value at the surface and hence the surface pH which is of prime importance.

The non-linear regression program, Modfit (McIntosh & McIntosh 1980) used the Marquardt (1963) algorithm. It was only slightly adapted to run on a PDP-11 computer. Convergence was rapid and the estimates were of acceptable precision. Average within data-set coefficients of variation were 8, 16, and 1·5% for k'_{12}, k'_{21} and m_i, respectively. These could be improved by increasing the precision of the assays and optimizing the sampling times.

The method described thus allows the kinetics of oral mucosal drug absorption to be studied in a single trial. Certain preliminary tests should be done to ensure that saliva

does not interfere with the drug and phenol red assays, that phenol red does not affect the absorption of the drug, and that the drug and phenol red do not interfere with each others assays. This method has two further advantages over the traditional buccal absorption test: it should allow pH changes to be incorporated into computations with certain precautions; and it should allow trials to be conducted in which disappearance form the mouth and appearance in the plasma are monitored and modelled simultaneously.

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