SESSION 3

Biopharmaceutics

119
Quality of chloroquine dosage forms in Lagos State General Hospitals, Nigeria

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Objectives To determine the quality of chloroquine dosage forms available in Lagos State General Hospitals (LSGHs), Nigeria. In Nigeria, malaria is a major cause of morbidity and it is still one of the major causes of hospital attendance (FMOH 2001). Until recently chloroquine has been the first line drug in treating malaria in Nigeria but it has been replaced due to therapeutic failure. Substandard or poor quality drugs can contribute to inappropriate dosage and treatment failure (Maponga & Ondari 2003).

Methods Fourteen different samples of chloroquine tablets, thirteen of syrups and five of injections were collected from all the LSGHs. British Pharmacopoeia (BP 2002) method was used to assess the following properties for tablets: uniformity of diameter, uniformity of weight, content of active ingredient, disintegration, dissolution, crushing strength (tablet 'hardness') and resistance to abrasion ('tablet friability'). Microbiological assay and determination of the content of active ingredient were carried out for syrups and injections using British Pharmacopoeia (BP 2002) method. Assay of active ingredient of tablet, syrup and injection was carried out by measuring the absorbance of the prepared solution of each dosage form at 343 nm using UV spectrophotometer. The concentration was then intrapolated from the standard calibration curve. This was done twice.

Results All the tablet samples passed the dissolution and disintegration tests according to British Pharmacopoeia (BP). Over 85% of the tablet samples complied with BP standard for the content of active ingredient. About 21% of the tablet samples failed the friability test. All the tablet samples passed the BP requirements of weight variation from mean weight, none deviated by 5% from mean weight. Over 90% of the syrup samples failed the BP standard for active ingredient. They had higher amounts than the BP standard. About 23% of the syrup samples failed the BP standard for microbial growth. These had more than 10^3 cfu/ml (*B. substilis*) but there was no growth of *Escherichia.coli, Staphylococcus aureus* and *Klebsiella* spp. All colonies stained showed a predominantly Gram Positive Large Bacilli indicative of the *Bacilus* spp. There was no growth in all the injection samples but all of them failed BP standard for active ingredient.

Conclusions From the results of the determination of the quality of chloroquine dosage forms obtained from the health facilities the tablets complied more with the BP standard in terms of active content than the other dosage forms. Tablet chloroquine might be a better option than syrup or injection. The tablets passed the quality tests more than the two other dosage forms. There is a need to determine the quality of chloroquine available in our hospitals. The quality of drugs being supplied to our hospitals should be verified and monitored.

Federal Ministry of Health (FMOH) (May 2001) National Malaria and Vector Control Division, Federal Republic of Nigeria National Antimalarial Treatment Policy

Maponga, C., Ondari, C. (2003) T*he quality of antimalarials. A study in selected African countries*. World Health Organisation. Geneva, WHO/EDM/PAR/2003.4, pp 1–54

120
The use of AFM to assess macromolecule suspension formulations for metered dose inhalers

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Objectives Within the pharmaceutical drug development sector, there is currently a growing interest in the pulmonary delivery of therapeutic proteins for systemic effect and plasmid DNA to correct genetic lung disorders. Both human insulin and salmon calcitonin have been incorporated into dry powder inhaler (DPI) formulations. However, whilst the pulmonary route of administration has proved effective for these therapeutic proteins, certain issues remain regarding the dose uniformity and dose efficiency of some formulation-device combinations. Despite these problems, neither human insulin nor salmon calcitonin has been incorporated into pressurised metered dose inhaler (pMDI) formulations. These proteins may be more stable in a closed pMDI environment rather than a DPI, where the formulation may be more exposed to environmental factors, such as moisture and air. As a result, this study sought to explore the adhesive behaviour of two therapeutic proteins (human insulin and salmon calcitonin) and a model protein (lysozyme) in suspension formulations for pMDIs, using Atomic Force Microscopy (AFM). The work also compares the behaviour of surfactant coated plasmid DNA.

Methods The adhesive forces of human insulin, salmon calcitonin, lysozyme (model) and surfactant coated plasmid DNA were acquired using AFM. The resultant force values, along with the calculated contact area were, where possible, used to determine the dispersive surface free energy of these macromolecules. AFM was also used to determine the adhesive force of each protein to several pMDI components in the presence of a model hydrofluoroalkane propellant (decafluoropentane).

Results Human insulin was found to have a considerably higher dispersive surface free energy than salmon calcitonin and lyszoyme. It was also found that human insulin displays higher adhesion to pMDI components than salmon calcitonin or lysozyme. Thus, the dispersive surface free energy of proteins may be a contributing factor in their adhesive interactions with various pMDI components. The type of surfactant used to coat the plasmid DNA particles was found, as expected, to have an effect on the surface energy. AOT-coated plasmid DNA was determined to have a lower surface energy than lecithin-coated DNA.

Conclusion The surface free energy and adhesive characteristics of three proteins (within a model pMDI system) have been determined using AFM. A clear link was found to exist between the dispersive surface free energy of each macromolecule and their corresponding adhesive interactions to various pMDI components. Thus, a knowledge of the surface free energy of each macromolecule, combined with their adhesive interactions within a pMDI system should be taken into consideration during the pre-formulation of such molecules. It allows for a greater understanding of the interactive relationships likely to exist within such systems. This study may also indicate that, whilst model proteins such as lysozyme tend to be used to assess potential macromolecule formulations, the results obtained may be of limited value.

121
A novel SPM-based approach to studying reagent-cell interactions

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Objectives The objective of this study is to develop a novel SPM-based approach whereby we are able to detect and quantify interactions between pharmaceutically relevant materials and biological cells. In the first part of this study, we have used AFM to develop a new protocol for imaging *Escherichia coli*. Secondly we describe the novel use of heated tip probes as a simple means of developing coated tips that are able to measure adhesive forces between the coating material and surfaces.

Methods *E. coli* bacterial cells were grown from overnight cultures at 35 °C with mild shaking at 150 rpm in Luria broth medium (tryptone 10 g l^{-1}, yeast extract 5 g l^{-1}, NaCl 5 g l^{-1}) to stationary growth phase. These were then harvested by centrifugation at 9000 rpm for 3 min, washed three times with PBS ((phosphate-buffer saline ($CaCl_2$, $MgCl_2$)). 50 μL of the cell suspension was placed on a glass slide coated with poly-L-lysine solution followed by incubation, rinsing and drying. The imaging of the cells was carried out in tapping mode using Veeco diCaliber AFM (Veeco Instruments Ltd, United Kingdom) and Pulsed Force Mode as these are the most suitable modes for the study of soft samples. Microthermal analysis was used to heat a probe in the presence of the material (in this case polyethylene glycol as a model) of interest and a small quantity removed via melting/softening such that the tip becomes contaminated. We have recently shown that it is possible to pick up femtograms of material using this approach (Harding et al 2007). Here we explore the use of this method to perform pull-off force measurements on a model system (PEG-coated tip on a surface of paracetamol) as proof of concept of the approach.

Results The sample preparation technique used differs from that described in previous studies in that we have used poly-L-lysine solution for immobilizing the cells. As a result we have obtained images of excellent resolution and are able to visualise specific regions of the cell surface. We have produced a series of force distance curves for uncoated and PEG-coated thermal probes; the uncoated probe showed no detectable hysteresis on approach and withdrawal while it is clear that the coated probe shows a distinct and quantitative adhesion to the surface, thereby demonstrating that the nanosampled tip is allowing measurement of adhesion of the coating material with the substrate of interest. Our ongoing studies are focused on measuring cell surface adhesion using probes coated with a range of recognized bioadhesive materials with a view to establishing a novel method of single cell interaction studies.

Conclusions Based on the above, we propose a simple, but effective and reproducible method for the preparation and imaging of *E. coli* bacterial cells. Chemical force microscopy based on tips coated using thermally assisted nanosampling has been shown to give clear results on model systems and this is currently being applied to the above biological cells.

Harding, et al (2007) *J. Pharm. Sci*. In press

122
Effect of natural bioenhancer on permeability of β blocker atenolol

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Objectives To increase the intestinal permeability and absorption of BCS Class III drug, atenolol, with use of herbal bioenhancer like piperine. Piperine has been reported and patented as to enhance the bioavailability (Majeed et al 1999). Atenolol is a hydrophilic and cardioselective β-blocker that is poorly absorbed in the lower gastrointestinal tract (50%). It is not metabolised and is excreted unchanged by the kidneys (Kirch & Gorg 1982).

Methods Three different methods were used to prepare the atenolol, physical mixture, solvent evaporation method and kneading method – piperine binary systems with ratios 25:5, 25:10, and 25:15. The binary mixtures were characterized by FTIR and DSC. The permeation study was conducted using goat intestinal membrane in the diffusion cell. The amount of drug permeated ($\mu g/cm^2$) was determined and plotted as a function of time (seconds). The permeability coefficients (P) were calculated from the linear part of the curves.

Results The IR spectrum and DSC data verifies that there are no alterations found in the characteristics of atenolol. This suggests physical characteristics of atenolol are not affected by piperine (Velpandian et al 2001). Permeation coefficient (P) was calculated for atenolol and binary systems. All three methods corroborate the increased permeation (Table 1) in comparison with plain atenolol.

Conclusions Piperine has enough potential to act as permeation enhancer. It is of herbal origin and also listed by FDA as Generally Recognised As Safe (GRAS). It can be utilized as the novel excipient without any toxicity on the intestinal mucosa. It excludes need for sophisticated and costly drug delivery system.

Table 1 Permeation coefficient of atenolol and binary systems with piperine

Atenolol: Piperine ratio	Permeation coefficient $P \times 10^{-4}$ (cm/sec) binary system (n = 4)		
Plain atenolol	0.51 ± 0.02		
	Physical mixture	Solvent evaporation method	Kneading method
25:5	0.62 ± 0.01	1.19 ± 0.03	0.83 ± 0.04
25:10	1.16 ± 0.02	0.58 ± 0.01	0.55 ± 0.02
25:15	0.83 ± 0.05	0.57 ± 0.03	0.53 ± 0.02

Kirch, W., Gorg, K. G. (1982) *Eur. J. Clin. Pharmacol*. **7**: 81–91
Majeed, M., et al (1999) *United States Patent No*. 5,972,382
Velpandian, T., et al (2001) *Eur. J. Drug Metab*. **26**: 241–247

123
Development and evaluation of Madin-Darby canine kidney (MDCK) monolayer permeability assay for profiling new chemical entities in early drug discovery

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Introduction Caco-2 is most widely used for prediction of intestinal absorption of potential drug candidates. But when taking a closer look, these cells reveal problems of long culture time (21 days) and sub-optimal standardization. MDCK cells provide a fast (3 day culture time) and more robust model for permeability due to advantages of easy handling, being less prone to external influences, with low expression of transporters and having little metabolic activity.

Objectives To develop and validate MDCK monolayer transport assay and to evaluate the utility of this assay against Caco-2, in determining permeability of new chemical entities (NCEs) in early drug discovery.

Methods Cell culture: Caco-2 (HTB-37) at passage 17 and MDCK (CCL-34) at passage 56 was obtained from ATCC (USA). Cells were cultured in DMEM supplemented with FBS, 1.0 mM sodium pyruvate, 0.1 mM non-essential amino acids, and 100 U/ml penicillin and streptomycin, grown in 5%CO_2 and 90% RH at 37 °C. Transport studies: Caco-2, cells were seeded onto 6-well polycarbonate inserts, 3.0-μm pore-size at a density of 3.0×10^5 cells/insert and grown for 21 days. In case of MDCK, 0.4-μm pore-size inserts seeded at a density of 10×10^5 cells/insert were maintained for 4 days. The integrity was determined by TEER value (>400 $\omega.cm^2$) and by leakage rate of lucifer yellow (< 1% per h) (Irvine et al 1999). The transport of drugs was examined by sampling at 30, 60, 90 and 120 min. MDCK assay is standardized using a set of six model drugs (whose absorption in humans range from 5% to 100%) and taking Caco-2 as a reference standard (Taub et al 2002). The validated assay is used to screen in-house new chemical entities belonging to three categories of anti-cancer agents viz. cyclopentanones, naphthyridines and betulinic acid derivatives. Analytical method: Rhodamine and doxorubicin were analysed fluorimetrically using Varioskan Instrument. Quantitation of remaining drugs was performed by an HPLC method using YMC, C-18, ODS-A RP column (250×4.6 mm, 4 μm) with acetonitrile and water (pH 3.0 adjusted with o-phosphoric acid) as a gradient at a flow rate of 1 ml min^{-1}.

Results The P_{app} values of six standard compounds in MDCK correlated well with Caco-2 (as seen in table below) and also with the reported percent absorption values in man. The data from standard drugs enabled in-house permeability classification as low ($<10 \times 10^{-6}$ cm s^{-1}), moderate ($10–100 \times 10^{-6}$ cm.s^{-1}) and high ($>100 \times 10^{-6}$ cm s^{-1}). In case of NCEs, cyclopentanones were the class of compounds with high permeability matching well with combretastatin A4. Naphthyridine series contained compounds which ranged from moderate to high permeability, whereas betulinic acid derivatives showed extremely poor permeability ($<1 \times 10^{-6}$ cm s^{-1}).

Conclusions The inclusion of MDCK assay in drug discovery process will expedite the identification of promising drug-leads. This system can predict successfully the rank order of permeabilities of NCEs useful in establishing structure-permeability relationships.

Irvine, J. D., et al (1999) *J. Pharm. Sci*. **88**: 28–33
Taub, M. E., et al (2002) *Eur. J. Pharm. Sci*. **15**: 331–340

124
The intrinsic dissolution rate of ketoconazole in proteinaceous biorelevant media

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Objectives The use of biorelevant media in drug dissolution was investigated in an attempt to develop a more realistic model to mimic what happens to a drug in the gastro-intestinal tract. In this investigation, the dissolution in proteinaceous biorelevant media was studied using the intrinsic dissolution rate technique (IDR), which studies the dissolution of a sample of compressed drug having a constant surface area which is in contact with the medium (USP 27, 2004). The media investigated were three types of milk of varying fat content, and which were mixed with Simulated Gastric Fluid (SGF, without enzymes) and four types of protein media chosen on the basis of their availability in food, such as albumin (hen egg white), gelatin (bovine skin), casein (bovine milk) and gluten (wheat).

Methods Ketoconazole was used as a model of a Class II drug according to the Biopharmaceutics Classification System (BCS) (i.e poorly soluble/highly permeable). The proteins were dissolved or dispersed in SGF and the final pH was adjusted to 3. Saturation solubility studies were carried out using the shake flask method. The IDR was performed by the stationary disc method, with 9.5 mm diameter discs prepared by compressing 150 mg of the drug in a die at 1000 psi. The drug was compressed against aluminium foil to produce smooth surface and the resulting discs were tested. A USP Type II dissolution tester (Pharmatest PTW S3C, Pharmatest GmbH) was used where experimental conditions consisted of 500 ml of dissolution medium at a temperature of 37 ± 0.5 °C and an agitation rate of 100 rpm. After appropriate time intervals, 1 ml samples were collected, basified and extracted by solvent extraction. The extracts were evaporated to dryness and dissolved in eluent prior to analysis by HPLC with UV detection.

Results The data obtained (Table 1) showed that milk increased the rate of dissolution of ketoconazole as compared with SGF but that the fat content of the milk appeared to have no effect. The presence of albumin and gelatin each increased the IDR whilst maintaining linear dissolution profiles and solubility whilst casein enhanced dissolution slightly but did not give linear dissolution profiles, and therefore an IDR could not be calculated. Gluten appeared to have no discernible effect.

Conclusions The mechanism behind the increased solubility and increased dissolution rates is unclear but might be attributed to protein-drug complexation or possibly micelle-formation (Macheras & Reppas 1987). The results highlight the potential importance of the effect of food on drug dissolution and consequent bioavailability, which critically underpin early clinical studies.

Table 1 Ketoconazole dissolution data in various media

Medium	IDR (mg/min/cm^2)	Amount dissolved in 60 min (mg/cm^2)
SGF pH 3	0.08	8.3
SGF:whole milk 1:1 $^{v}/_{v}$	0.39	27.5
SGF:semi-skimmed milk 1:1 $^{v}/_{v}$	0.42	25.4
SGF:skimmed milk 1:1 $^{v}/_{v}$	0.32	23.3
Albumin 1% w/v	0.19	13.5
Gelatin 1% w/v	0.25	17.8
Casein (Saturated solution)	—	11.7
Gluten (saturated solution)	0.08	8.0

Macheras, P., Reppas, C. (1987) *Int. J. Pharm*. **37**: 103–112
United States Pharmacopoeia 27 (2004) Rockville: United States Pharmacopoeial Convention Inc.

125
Evaluation and comparison of alternative dissolution tests in the development of chewable tablet for short-term treatment of the oesophageal infection

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Objectives Localised delivery of antifungal agents to the oesophagus offers benefits over systemic therapy including lower doses of drugs. Chewable tablets that release drug into the saliva can be used as a reservoir for oesophageal drug delivery, where concomitant release of the viscosity-enhancing agent with drug leads to drug within viscous saliva which increases oesophageal transit time and assists in local delivery to the oesophagus. In this study, miconazole chewable tablets were developed for the local therapy of oesophageal infection commonly associated with immunocompromised patients.

Methods Chewable tablets (1050 mg/tablet) were prepared by direct compression. Whipsol H15 (hard fat) was used as matrix base due to its melting temperature being close to the temperature of the oral cavity, and a 'smooth melting feeling' during chewing. Sodium alginate was selected as viscosity-enhancing agent to increase the viscosity of the saliva as drug is released to prolong the transit time of drug through the oesophagus providing increased local contact. The release medium in all cases was 1%w/v SDS in PBS buffer at pH 7.4 maintained at 37 ± 0.5 °C. Miconazole was used as a model drug quantified via HPLC. As shown in Table 1, the tablets were tested as whole tablet (Basket A) or similar size pieces of crushed tablet (Basket B), respectively, using rotating basket method. Paddle method (Paddle A) with rotating paddle alone was also modified with additional glass beads (Paddle B) aim to more agitation. Drug release was also measured using specific apparatus for chewing gums described in European Pharmacopoeia 2004, which simulates human chewing behaviour.

Results Table 1 showed the drug release comparison of the chewable tablet containing 500 mg sodium alginate, 500 mg Whipsol H15 and 50 mg miconazole by the alternative dissolution tests. All chewable tablets using chewing apparatus showed complete drug release within 60 min with no measurable lag period

before drug release. When the chewable tablets were tested by rotating basket method at 50 rpm, the crushed tablet as pieces showed faster drug release than the whole tablet; however, both cases showed linear, zero-order release. In paddle methods, additional glass beads enhanced the drug release rate in comparison with paddle alone. The glass beads have been recommended by Siewert et al (2003) within the dissolution medium for a large amount of agitation to improve the drug release rate.

Conclusions Preliminary observations suggest that the drug release behavious of the chewable tablets may be altered depending upon the in vitro dissolution method examined. This study demonstrated the needs of modified drug release apparatus for chewable tablets. A comparison to in vivo release would assist in selection of the most appropriate in vitro model.

Table 1 release rate using different dissolution apparatus

Dissolution apparatus	**% Drug released**			
	30 min	60 min	90 min	180 min
Chewing apparatus	60	100	100	100
Basket A	0.86	1.7	5.4	18.6
Basket B	4.4	8.3	11.3	27.9
Paddle A	22.6	37.6	41.1	59.7
Paddle B	33.6	73.2	88.1	100

Siewert, M., et al (2003) *AAPS PharmSci* **2**: 6–15