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Characterization of silicone elastomer vaginal rings containing HIV microbicide TMC120 by Raman spectroscopy

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Abstract

Silicone elastomer vaginal rings are currently being pursued as a controlled-release strategy for delivering microbicidal substances for the prevention of heterosexual transmission of HIV. Although it is well established that the distribution of drugs in delivery systems influences the release characteristics, in practice the distribution is often difficult to quantify in-situ. Therefore, the aim of this work was to determine whether Raman spectroscopy might provide a rapid, non-contact means of measuring the concentrations of the lead candidate HIV microbicide TMC120 in a silicone elastomer reservoir-type vaginal ring. Vaginal rings loaded with TMC120 were manufactured and sectioned before either Raman mapping an entire ring cross-section (100 μm resolution) or running line scans at appropriate time intervals up to 30 h after manufacture. The results demonstrated that detectable amounts of TMC120, above the silicone elastomer saturation concentration, could be detected up to 1 mm into the sheath, presumably as a consequence of permeation and subsequent reprecipitation. The extent of permeation was found to be similar in rings manufactured at 25 and 80°C.

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Introduction

This study is concerned with spectroscopic characterization of reservoir-type vaginal rings, which are presently being developed as controlled-release devices for long-term administration of vaginal microbicides to prevent heterosexually acquired HIV. In particular, the aim is to develop an understanding of changes in distribution of the lead candidate, vaginal microbicide TMC120, within a ring formulation following the manufacturing process by carrying out in-situ Raman studies.

The advantages of using Raman spectroscopy for the analysis of pharmaceutical solid dosage forms have been thoroughly documented (Williams 2001). The spectra can be recorded with no sample preparation and each individual component gives rise to a unique set of bands (Bell et al 2000). The richness of the spectra means that quantitative data analysis, either by manually selecting and then measuring some of the bands due to each of the components or by using multivariate data analysis techniques (for example partial least squares (PLS)), is straightforward. The fact that the scattering is generated by a focussed laser gives an inherent spatial resolution and this can be exploited in Raman mapping experiments, which allow Raman chemical images to be obtained. These images show the distribution of the various constituents within solid dosage forms (typically the active pharmaceutical substance and excipients in tablets). They are most often implemented in micro-Raman systems where small areas are mapped at very high spatial resolution ($\geq$1 μm) (Clarke et al 2001). However, use of micro-Raman instruments is inappropriate if the features of interest are on much larger length scales. It is possible to record data on sampling grids where the spacing between the probed areas is very much larger than the probed areas, but this always carries the risk of sampling error. For these systems the optimum solution is to use a macro system, where the spot size is deliberately increased to match the spacing needed to sample macroscopic regions of the dosage form (Bell et al 2004). In the case of the vaginal rings studied here the length scales of interest were hundreds of μm so a

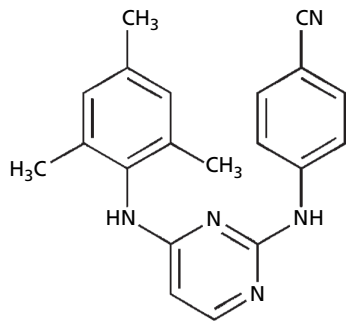
"macro" Raman system with 100 μm analysis spot diameter was the most appropriate.

Vaginal microbicides are currently being developed as a preventative strategy for HIV infection. These are chemical substances that, when formulated appropriately and applied to the vagina before intercourse, have the potential to either prevent or reduce the risk of HIV transmission through a number of well-established mechanisms (Malcolm et al 2004). As of June 2005, 15 HIV microbicide candidates, including the potent experimental non-nucleoside reverse transcriptase inhibitor (NNRTI) TMC120 (see Figure 1), were undergoing clinical trials. Most of these compounds have been evaluated in conventional semisolid gel formulations designed to provide a single dose of the microbicidal agent, applied immediately before every act of intercourse. However, long-term administration of vaginal microbicides using controlled-release formulations may overcome many of the compliance, acceptability and efficacy issues associated with single-dose, gel-based products. Several in-vitro and animal model studies have demonstrated that TMC120 is a potent antiretroviral that effectively prevents both cell-free and cell-associated HIV infection at concentrations in the nanomolar range. In addition, the low cytotoxicity and high therapeutic index of TMC120 make it an ideal candidate for development as an HIV vaginal microbicide. Previous in-vitro release studies have demonstrated that TMC120 may be continuously released from a single silicone core-type vaginal ring in the daily microgram range over a period of at least 70 days and potentially for up to four years (Malcolm et al 2005).

Materials and Methods

Materials

TMC120 was kindly provided by the International Partnership for Microbicides (Maryland, US). MED6382 medical grade silicone elastomer was supplied by Warner Chilcott, UK. Tetrapropyl orthosilicate (95%) and tin(II) 2-ethylhexanoate (95%) were purchased from Sigma–Aldrich (Gillingham, UK).



TMC120

Figure 1 Structure of TMC120.

Manufacture of core-type silicone vaginal rings loaded with TMC120

Core-type vaginal rings (also known as 'reservoir-type' rings) containing 400 mg TMC120 in a full-length silicone elastomer core and encapsulated with a non-medicated silicone elastomer sheath layer were manufactured on a laboratory-scale ring-making machine according to a standard method already described in the literature (Malcolm et al 2005). The rings had the following characteristics: 5.5-mm core cross-sectional diameter; 9.0-mm ring cross-sectional diameter; 55.0-mm overall ring diameter; 10.0 (+0.2) g mean weight of rings.

Raman spectroscopy

TMC120-loaded core-type vaginal rings were prepared for analysis by cutting approximately 2-mm thick cross-sections from the rings and attaching to a glass microscope slide using a small drop of cyanoacrylate adhesive.

Raman spectra were recorded on an Avalon Instruments RamanStation fitted with a long travel, precision motorized x-y-z sample translation stage driven under software control. The system has a 785-nm diode laser excitation source (approx. 100 mW, 100 μm beam diameter at sample) and an echelle spectrometer/cooled CCD detector combination which allowed the entire Raman spectral range to be recorded in a single accumulation.

Raman maps were recorded with accumulation times of 1 s per point and were subsequently analysed using the instrument's "Insight" software suite. Line scans were recorded in kinetic mode in which the system was set to automatically drive to a series of points at 100 μm spacing across the diameter of the sample and to record spectra (accumulation time 120 s) at each point. The system was set up to repeat this scan sequence at 20-min intervals for the first 5 h of accumulation and at 1-h intervals up to 30 h.

Results and Discussion

Figure 2 shows the Raman spectra of the major pre-manufacture constituents of the vaginal rings along with spectra of the TMC120-loaded silicone elastomer core. The spectrum of the intravaginal ring (IVR) core showed the characteristic bands of the silicone elastomer, the strongest of which was the CH_3 symmetric stretch at 2906 cm^{-1} (compared with Figure 2 (a)) and of the diatomaceous earth mechanical filler, which gave the broad feature around 1400 cm^{-1} that underlayed the much narrower silicone bands and was apparent in Figure 2 (b). The spectrum of the core showed additional strong bands due to the TMC120 (e.g. the CN stretching band 2223 cm^{-1}) whose spectrum in the silicone matrix was identical (within experimental error) to that of the pure drug (Figure 2 (c)).

The relative band intensities of silicone elastomer and drug could be used to quantify drug content in the ring. Initial experiments showed that the drug to elastomer ratio was not constant across the core, although it was clear that the average concentration within the core was very much higher than

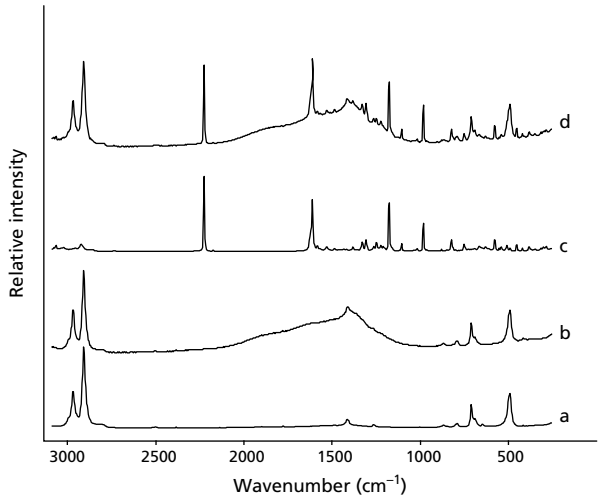


Figure 2 Raman spectra of (a) the silicone elastomer used in the IVR preparation, (b) silicone elastomer with 22% diatomaceous earth added, (c) TMC120, and (d) the core of a typical reservoir type IVR.

that within the sheath. Figure 3 shows a Raman chemical image of a typical cross section through both core-type (Figure 3A) and matrix-type (Figure 3B) vaginal rings. In a matrix-type ring, the TMC120 was distributed throughout the ring. These Raman maps illustrate data from approximately 20 000 individual spectra. The false colour scale was determined from principal component analysis of the raw data and it reflected the value of the most significant factor at each x-y coordinate. One obvious advantage of the Raman mapping was that the data in Figure 3A for example allowed the extent of the core to be clearly delineated. It was difficult to identify the core/sheath boundary optically, particularly with low loadings, since there was no obvious sharp change in colour or opacity that could be observed under low power microscopy, but the Raman data allowed the points where there was a marked change on drug concentration to be identified. Throughout this work this chemical change was taken to indicate the effective outer boundary of the core, although of course strictly speaking such points marked the limits of heavily medicated regions rather than the boundary between the elastomeric core and sheath. Also shown in Figure 3B is the Raman chemical image of a matrix-type ring. In this sample the chemical image showed ~mm diameter domains of high drug concentration surrounded by an unmedicated silicone elastomer matrix.

The solubility of TMC120 within the elastomer was extremely low (3.7 μg mL^{-1}, unpublished data) so we would not expect to be able to detect dissolved drug within the silicone, even at near-saturation levels. Consistent with this, it was found that even the strong 2223 cm^{-1} drug band was not detectable in the spectra of a saturated solution of TMC120 in silicone oil, which was used as a proxy for the silicone elastomer (data not shown). This meant that any spectra with discernible drug bands were displaying evidence for solid drug that was present at concentrations well above the saturation value and which constituted a reservoir of undissolved drug in the ring. Since the ring was manufactured in a two-stage process, where the core was loaded with excess solid drug and then surrounded by an non-medicated sheath in subsequent steps, it might have been expected that a line scan across the diameter of a ring would have a "top hat" profile with very high concentrations in the core falling sharply to the very low saturation concentration which would be established within the sheath. Figure 4 shows data taken from line scans across a core-type vaginal ring section; the data have been truncated to allow the interface between the core and sheath to be shown in detail. The plot shows the relative intensity of the strongest silicone to drug bands, which will be directly proportional to their relative concentrations. As

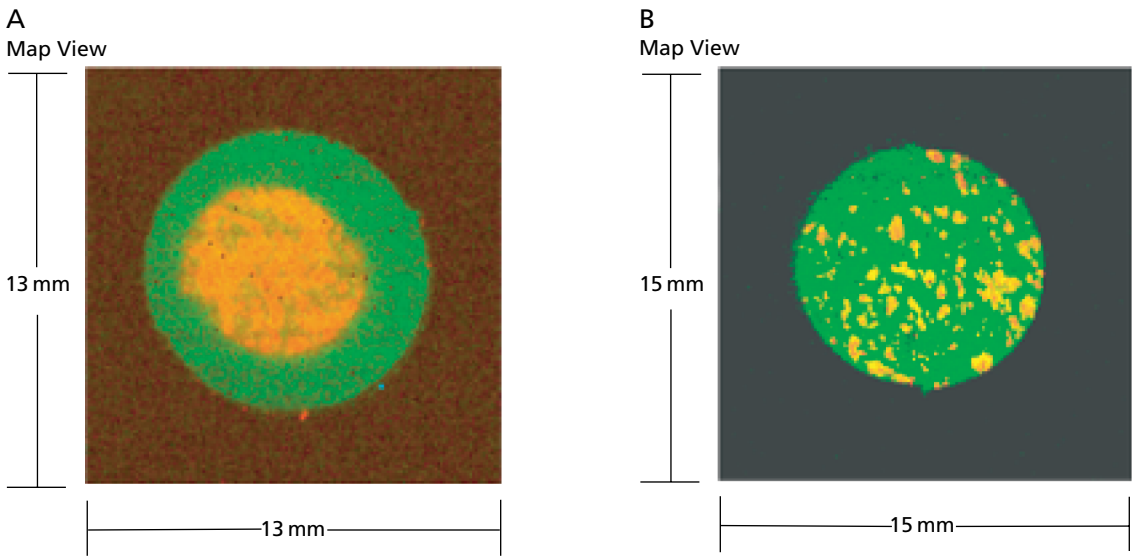


Figure 3 Raman chemical images of cross-sections through a typical core-type (A) and matrix-type (B) vaginal ring. These Raman maps illustrate data from approximately 20 000 individual spectra. The false colour scale was determined from principal component analysis of the raw data and it reflects the value of the most significant factor at each x-y coordinate.

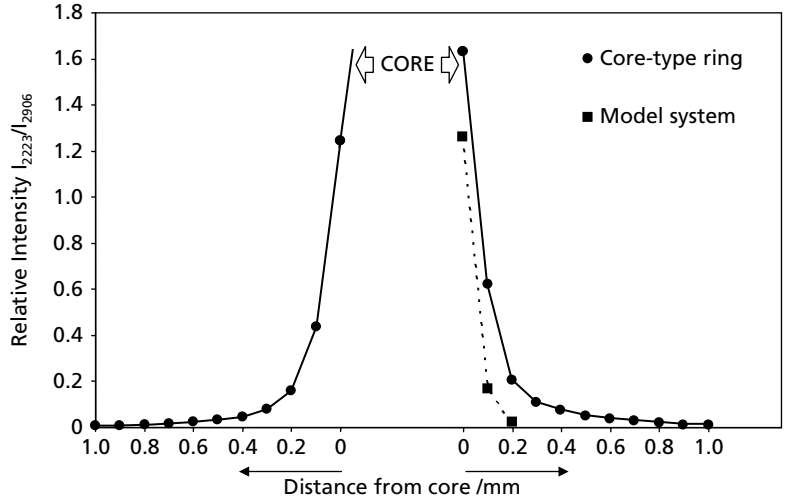


Figure 4 A plot of the relative intensity of the strongest silicone to TMC120 Raman bands as a function of distance in a core-type vaginal ring. The data have been truncated to allow the interface between the core and sheath to be shown in detail. Also shown are data from a model system which had the same optical properties but an infinitely sharp fall off in drug concentration.

discussed above, the limit of the core was defined as the point after which the drug concentration showed a significant drop, but it was clear from the data that the intensity of the drug band fell relatively gradually with distance on moving from core to sheath. Since a single line scan allowed two boundaries to be probed, the fall off with distance on diagonally opposite sides of the ring could be compared under identical accumulation conditions and the data showed similar fall off at each side. The gradual fall in drug concentration at the boundary was not an experimental artifact; Figure 4 also shows data from a model system which had the same optical properties but an infinitely sharp fall off in drug concentration. This model was created by mounting two flat elastomer sections, one cut from the core and one unmedicated, in close physical contact. It was clear from Figure 4 that the drug signal decreased much more rapidly with distance in the model than in the vaginal ring.

The silicone used to prepare the sheath layer initially had no TMC120 present so the solid drug detected in the Raman experiments must have been due to drug moving into the sheath either during manufacture or during storage. To determine if drug diffusion occurred in the first hours following manufacture, repeated line scans of the same sheath were run from approximately 2-h post-manufacture up to 30 h. Figure 5 shows spectra

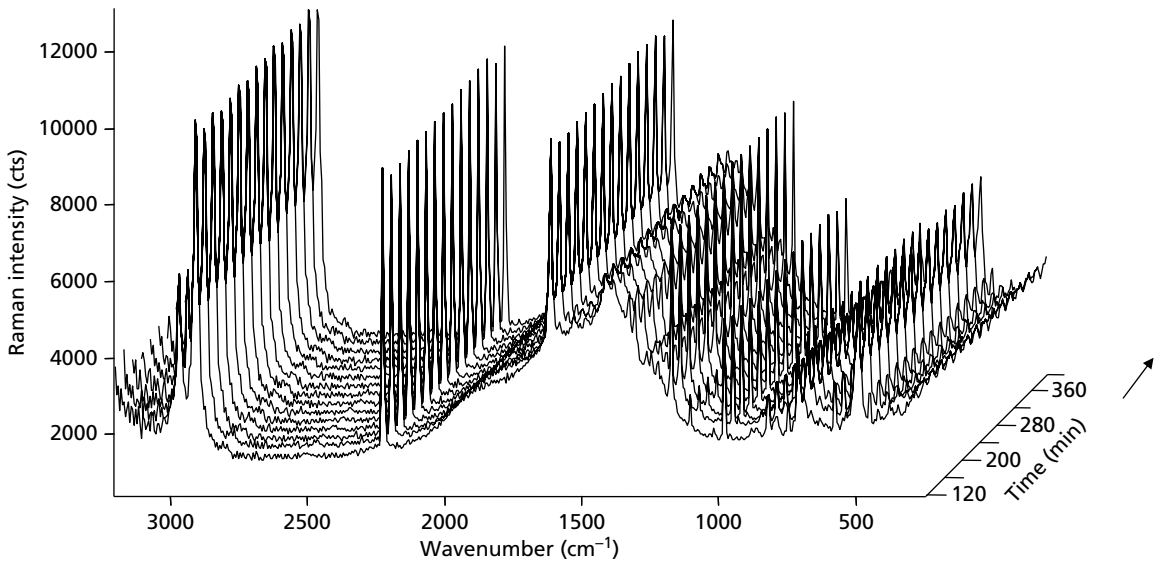


Figure 5 Raman spectra of a core-type IVR taken at 20-min intervals from 120- to 400-min post-manufacture. Data were extracted from line scans but show spectra where the sample stage had returned to the same x-y position each time.

of the same x-y position extracted from line scans taken at 20-min intervals from 120 to 400-min post-manufacture. This x-y position was chosen because the strongest silicone and drug bands had similar intensity at this point, which meant that any change in the relative proportions of each would be immediately apparent. It was clear from the data that the relative intensity did not change over the time period, apart from small random variations which resulted from uncertainties in repositioning; although these were < 1% of the scan range they could still be detected due to the rapid change in drug content per unit distance in these samples. Indeed, no change was detected 2–30 h after manufacture, which implied that drug migration into the sheath occurred either during manufacture or in the 2 h immediately post-manufacture. An obvious possibility was that the elevated curing temperature during manufacture increased drug solubility within the elastomer leading to increased permeation of solubilized drug into the sheath, but then results in precipitation of the drug in the sheath as temperature and solubility subsequently decreased. Figure 6 compares concentration profiles at the core sheath boundary of IVRs, when ring manufacture was carried out at 80 and 25°C. The data were plotted on log scales to allow better comparison and again data from opposite sides of the core were plotted to show the experimental uncertainty. It was clear that room temperature manufacture had little impact on the extent to which the drug migrated into the sheath, which implied that manufacture at elevated temperature was not the principal cause of the drug migration. Other possible sources of migration included the solubilization and migration of TMC120 from the core to the sheath in the propanol condensation product formed during curing of the silicone elastomer.

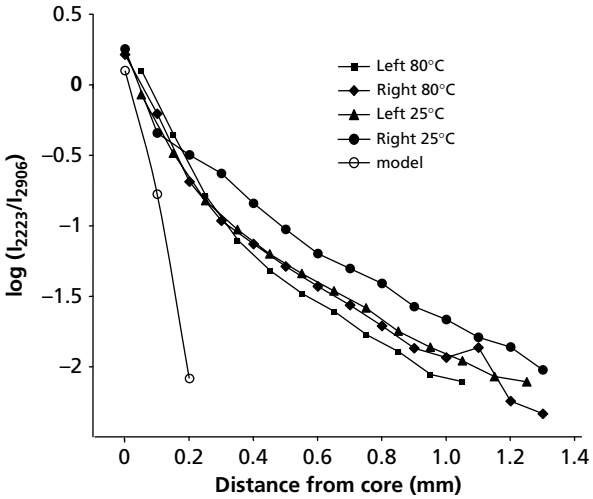


Figure 6 A plot of the relative intensities of the strongest silicone to TMC120 Raman bands as a function of distance in two core-type vaginal rings, one manufactured at 80°C and one at 25°C. The data have been truncated to allow the interface between the core and sheath to be emphasized and are plotted on a semi-log scale. Data from opposite sides of the cores have been plotted on the same axes to give an indication of the experimental uncertainty and measurements taken from a model system with an infinitely sharp concentration drop have been included for comparison.

Irrespective of the detailed mechanism of formation it was clear that Raman spectroscopy provided a simple and rapid method for characterization of TMC120 distribution with silicone elastomer core rings, and is likely to be applicable to other controlled-release polymeric drug delivery systems containing a Raman-active drug substance.

Conclusions

Vaginal rings have already been commercialized as controlled-release drug delivery systems for hormone replacement and contraceptive therapies. Presently, new ring formulations are being developed for the controlled release of antiretroviral substances as a preventative microbicidal strategy for HIV. The distribution of active agents within ring formulations is known to modify the drug release characteristics, although there are no simple, analytical methods for quantifying drug distribution throughout the polymer matrix. We have shown that Raman spectroscopy could be used to determine the distribution of a lead HIV microbicide candidate non-nucleoside reverse transcriptase inhibitor, TMC120, in both the sheath and core of a silicone elastomer reservoir-type vaginal ring since the active and the various components that constitute the silicone matrix gave distinct spectral features. Relative band intensities could be used to determine the relative concentrations of each that were present at any given point. It was found that detectable concentrations of TMC120 were present in the initially drug-free sheath, but that the extent of penetration into the sheath was independent of the ring manufacturing temperature.

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