International Journal of Pharmaceutics 548 (2018) 689–697

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Solid state ^{13}C NMR spectroscopy provides direct evidence for reaction between ethinyl estradiol and a silicone elastomer vaginal ring drug delivery system

Clare F. McCoy[a], David C. Apperley[b], Bruce Variano[c], Heather Sussman[c], Daniel Loeven[c], Peter Boyd[a], R. Karl Malcolm[a,*]

[a] School of Pharmacy, Queen's University Belfast, Belfast BT9 7BL, UK
[b] EPSRC National Solid-State NMR Service, Durham University, Durham DH1 3LE, UK
[c] Population Council, One Dag Hammarskjold Plaza, New York, NY 10017, USA

ARTICLE INFO

Keywords:
Covalent binding
17α-Ethinyl-estradiol
^{13}C-labelled
Addition cure silicone elastomer
Nuclear magnetic resonance
Hydrosilylation

ABSTRACT

Steroid molecules have a long history of incorporation into silicone elastomer materials for controlled release drug delivery applications. Previously, based on *in vitro* release testing and drug content analysis, we demonstrated indirectly that the contraceptive progestin levonorgestrel (LNG) chemically and irreversibly binds to addition cure silicone elastomers, presumably via a hydrosilylation reaction between the levonorgestrel ethynyl group and the hydrosilane groups in the poly(dimethylsiloxane-co-methylhydrosiloxane) crosslinker of the silicone elastomer. Here, for the first time, we report that solid state ^{13}C nuclear magnetic resonance (NMR) spectroscopy provides direct evidence for the irreversible binding of ethinyl estradiol (EE) – an estrogenic steroid molecule also containing an ethynyl functional group – to an addition cure silicone elastomer. By preparing silicone elastomer samples containing ^{13}C-labelled EE, signals in the NMR spectra could readily be assigned to both the free and bound EE. Additional depolymerisation studies, performed on an addition cure silicone elastomer system from which the unbound EE fraction was completely extracted, further confirmed the presence of bound EE through the formation of coloured reaction mixtures resulting from the reaction of bound EE and trifluoroacetic acid (TFA). These methods will be particularly useful in the ongoing development of new steroid-releasing silicone drug delivery devices, including various vaginal ring devices for contraception, HIV prevention and multipurpose prevention technology applications.

1. Introduction

Following first demonstration in 1966 that steroid molecules could permeate through the walls of silicone rubber devices implanted subdermally in ewes (Dziuk and Cook, 1966), a number of steroid-releasing silicone elastomer controlled release drug delivery devices have since reached market, including the subdermal implants Norplant®, Norplant II® and Jadelle® (Croxatto, 2002), the levonorgestrel-releasing intrauterine device Mirena® (Rose et al., 2009; Mansour, 2012), and the vaginal ring products Estring®, Femring®, Progering® and Fertiring® (Ballagh, 2004; Friend, 2015; Nath and Sitruk-Ware, 2010; Merkatz et al., 2009; Buckler and Al-Azzawi, 2003; Al-Azzawi and Buckler, 2003). Several new silicone elastomer vaginal rings are currently in development, including a dapivirine (DPV)-releasing ring for prevention of infection with the human immunodeficiency virus (HIV) (Baeten et al., 2016; Nel et al. 2016, 2009; Malcolm et al., 2016; McCoy et al., 2017), a combination dapivirine/maraviroc vaginal ring for HIV prevention (Fetherston et al., 2013; Chen et al., 2015), a dapivirine/levonorgestrel (LNG) ring for combination HIV prevention and contraception (Boyd et al., 2016; Murphy et al., 2016), a combination anastrozole/LNG ring as a novel therapy for treatment of endometriosis (Reinecke et al., 2016; Rotgeri et al., 2015; Schultze-Mosgau et al., 2016), a vaginal ring releasing the progesterone receptor modulator ulipristal acetate for contraception (Huang et al., 2014; Brache et al., 2012), and a Nestorone® (NES; segesterone acetate)/ethinyl estradiol (EE) ring for combination contraception (Weisberg et al., 2005; Fraser

Abbreviations: $CDCl_3$, deuterated chloroform; CP, cross-polarisation; DAC, dual asymmetric centrifuge; DPV, dapivirine; EE, Ethinyl estradiol; EE-$^{13}C_2$, 17α-ethinyl-$^{13}C_2$-estradiol; HIV, human immunodeficiency virus; LNG, levonorgestrel; NES, Nestorone; NMR, nuclear magnetic resonance; ^{13}C-ssNMR, solid-state ^{13}C nuclear magnetic resonance spectroscopy; TOSS, total suppression of spinning sidebands; TFA, trifluoroacetic acid

* Corresponding author.
E-mail address: k.malcolm@qub.ac.uk (R.K. Malcolm).

https://doi.org/10.1016/j.ijpharm.2018.07.040
Received 23 March 2018; Received in revised form 25 June 2018; Accepted 14 July 2018
Available online 20 July 2018
0378-5173/

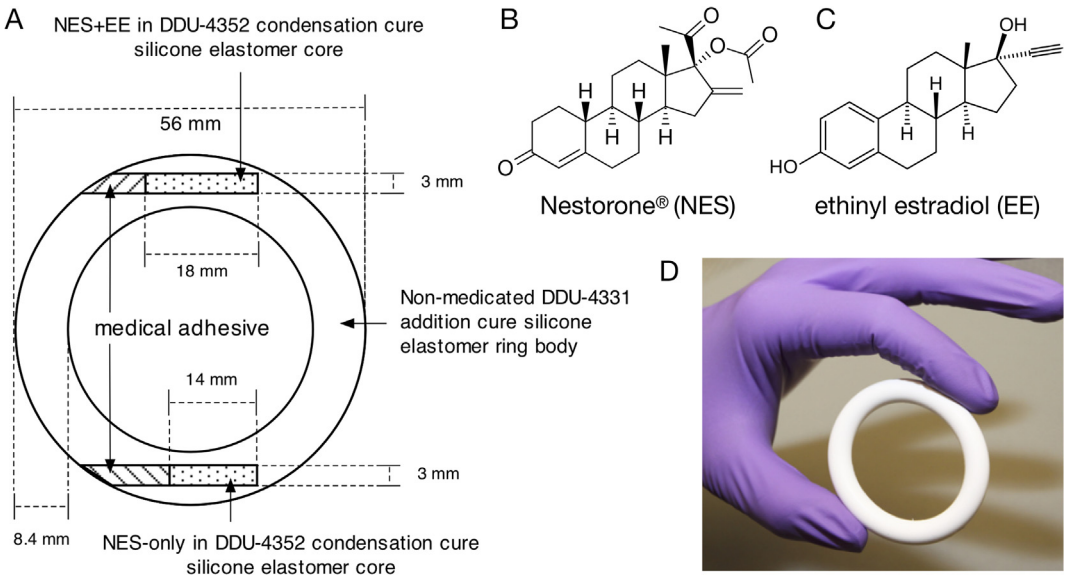

Fig. 1. A – Schematic of NES/EE contraceptive ring showing dimensions and location of drug-loaded cores; B – chemical structure of the progestin Nestorone®; C – chemical structure of the estrogen ethinyl estradiol; D – photograph of NES/EE vaginal ring.

et al., 2005; Sivin et al., 2005; Croxatto et al., 2006; Jensen et al., 2018; Merkatz et al., 2014; Stifani et al., 2018). Given the limited choice of medical grade polymers for long-term human implantation, silicone elastomers will likely remain an important material for development of controlled release drug delivery devices (Mashak and Rahimi, 2009; Kleiner et al., 2014; Malcolm et al., 2016).

Recently, as part of preclinical development of the DPV/LNG vaginal ring, we reported the irreversible binding of LNG, but not DPV, in the addition cure silicone elastomer material used to construct the ring, resulting in a reduction in the content assay value of LNG and impacting LNG *in vitro* release (Murphy et al., 2016; Boyd et al., 2016). Despite a lack of direct evidence, we hypothesized that the binding involved a hydrosilylation reaction between the LNG ethynyl group (C≡C) and the hydrosilane (Si−H) functionalised polydimethylsiloxane molecules within the elastomer system (Lidegaard et al., 2012; Lewis et al., 1991), rendering the LNG covalently attached to the elastomer and incapable of release (Murphy et al., 2016). During the normal silicone elastomer curing process, these hydrosilane groups react with vinyl-functionalised (Si−CH=CH_2) polydimethylsiloxane molecules (Fig. 2). The degree of LNG binding could be reduced through modification of the silicone cure temperature and time and by using larger LNG particles (Murphy et al., 2016). However, direct evidence for the covalent binding reaction could not be confirmed using nuclear magnetic resonance (NMR) analysis as the bound LNG fraction in the silicone elastomer was below the level of detection.

The NES/EE vaginal ring, currently under development by the Population Council (New York, USA), comprises a silicone elastomer ring body into which two steroid-containing silicone elastomer cores are inserted – one core contains only NES and the second both NES and EE (Fig. 1). The drug-free rate-controlling ring body is manufactured from an addition cure silicone elastomer while the drug-loaded cores are prepared using a condensation cure silicone elastomer (Fig. 1A). Other drug delivery devices are similarly constructed using both drug-loaded silicone elastomer cores and drug-free silicone elastomer sheaths, such as the LNG-releasing Mirena® intrauterine device (Robertson and Braun 1982) and the Jadelle® subdermal implant (Sivin et al., 2001; Croxatto, 2002). The condensation and addition cure chemistries of these silicone elastomer systems are very different. Condensation cure silicones are often preferred for incorporation of drugs due to their greater compatibility with a wider range of chemical functional groups than addition cure systems (Malcolm et al., 2016). However, the condensation cure reaction produces propanol (or other volatile alcohols) as a reaction by-product, which has a tendency to dissolve the incorporated drug and lead to a large initial burst of release (Malcolm et al., 2012). In contrast, addition cure silicone elastomer systems, which do not produce any alcoholic reaction product, are susceptible to deactivation of the platinum catalyst by certain chemical functional groups leading to inhibition of cure (Jerschow, 2001). During stability testing of the NES/EE vaginal ring, a lower-than-expected content assay value for the EE component in the ring device was measured, similar to that observed previously for the LNG component in the DPV/LNG ring. It is notable that both EE and LNG contain the same ethynyl functional group appended to the same carbon of the D ring of the steroid structure. Since EE could be fully recovered via solvent extraction from NES + EE cores that had not yet been assembled into rings (even after long term storage), the reduced EE assay value was tentatively attributed to a hydrosilylation reaction between EE and the hydrosilane groups in the addition cure silicone elastomer ring body upon molecular permeation of EE through the ring body; the same reaction as reported previously for LNG in the DPV/LNG ring. To date, no direct evidence for this reaction has been reported.

Here, we report that solid state ^{13}C nuclear magnetic resonance spectroscopy (^{13}C-ssNMR) provides the first direct evidence for irreversible binding of EE to an addition cure silicone elastomer. By preparing silicone elastomer samples containing ^{13}C-labelled ethinyl estradiol (specifically, the ethynyl carbons are labelled and are therefore particularly sensitive to any reaction at this site), signals in the ^{13}C-ssNMR spectra could readily be assigned to both the free and bound steroid.

2. Materials and methods

2.1. Materials

Addition cure silicone elastomer systems DDU-4331 (also known as MED4-4224) and DDU-4320 were supplied by NuSil™ Technology LLC (Carpinteria, CA, USA). Micronised ethinyl estradiol (EE) was supplied by Bayer AG (Bergkamen, Germany). Non-micronised 17α-ethinyl-$^{13}C_2$-estradiol (20,21–$^{13}C_2$ labelled; 99.1% isotopic enrichment) (EE-$^{13}C_2$) was purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). Particle size reduction of EE-$^{13}C_2$ was achieved by manual grinding in a mortar and pestle. Deuterated chloroform ($CDCl_3$, 99.8 at