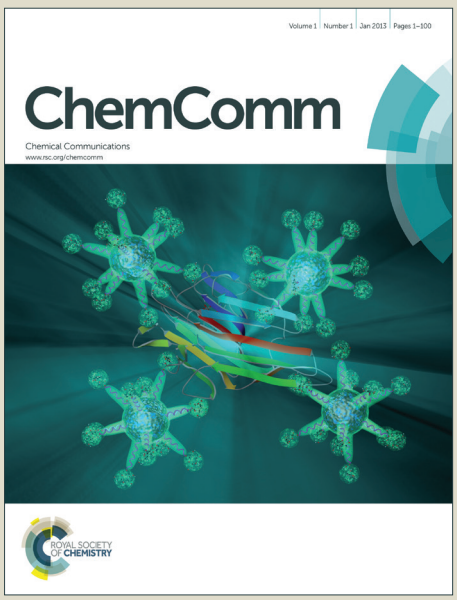
ChemComm

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

Journal Name

RSCPublishing

COMMUNICATION

Controlled and sustained release of pharmaceuticals via a single step solvent-free encapsulation

Cite this: DOI: 10.1039/x0xx00000x

A. Cavallaro[a] and K. Vasilev[b*]

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

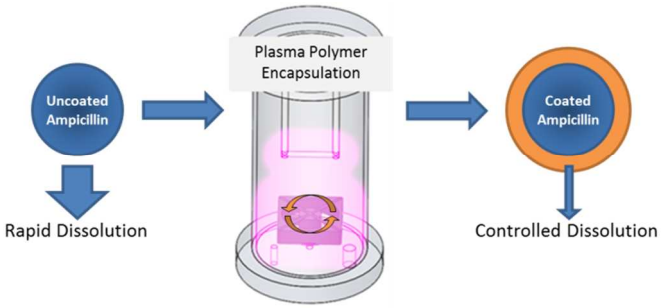
Herein, we report a novel and solvent-free technique for the encapsulation of pharmaceuticals that allows achieving controllable release rates. The method utilizes the deposition of a plasma polymer coating of controlled chemistry and thickness on the outer surface of drug particles placed under continuous agitation.

Today, the benefits of using controlled drug release systems in medical therapies are well recognized.[1] In conventional drug administration, the substance is administered in high doses only to be repeated hours or a day later. The drawbacks of this approach for the patient are high costs and possible systemic toxicity. In addition, many patients have issues with compliance to the administration regime.[2] These are some of the reasons that brought into light the concept of controlled release. Over the last decades, a vast number of technologies have been proposed to provide controlled, targeted and sustained release of pharmaceuticals delivered by oral or intravenous administration, via specifically designed carriers or implantable devices.[1b, 3] Most of the technologies involve polymeric systems which entrap the drug into capsules or into surface coatings. The preparation of such formulations require wet processes which demand multistep processing and solvents (such as LBL and miniemulsion techniques) that have a high cost and often limited encapsulation efficacy.[4] The use of nanoparticles and liposomes as drug carriers has also been extensively investigated.[5]

In this communication, we report a facile, one step and dry process for encapsulation and control of the release rate of pharmaceuticals. Importantly, as demonstrated in this report, the technology can be applied to "off the shelf" commercially formulated compounds without the need for any pre-treatment steps. The process is based on plasma polymer encapsulation of drug powders placed on a moving platform that provides random motion of the particles. A schematic of the experimental approach is shown in the Scheme. 1 and Fig. S1 of the supplementary information. We selected ampicillin as model drug to demonstrate the technology for two reasons. Firstly, ampicillin is a well-established, often prescribed broad-spectrum antibiotic commonly used in pharmacokinetic and antibiotic resistance studies, which makes the compound an excellent model drug. Secondly, providing advances into the delivery of antibiotics allows us to make a contribution towards easing the mortality and morbidity caused by infectious diseases: the second leading cause of death after heart diseases.[6]

Antibiotics have previously been used with plasma polymers with the purpose of generating antibacterial coatings. Examples are those loaded into the plasma polymer films[7] or into porous materials.[8] Covalent attachment to plasma polymer coatings has also been reported[9].

However, this is the first report that demonstrates encapsulation of individual antibiotic particles via plasma polymers. The goal is to achieve a sustained and controlled release of concentrations above the minimum inhibitory concentration (MIC) for a period of at least 5 days. Since ampicillin is highly hydrophilic, as many common antibiotics, we chose a plasma polymer deposited from vapour of 1,7-octadiene (ppOD) as the encapsulating material. The resulting coatings are relatively hydrophobic (Fig. 1b) and hence we hypothesised that this type of encapsulation will reduce the interactions between the drug and aqueous medium. In addition, 1,7-octadiene is easy to deposit from plasma and there are published evidence that the coatings are not cytotoxic.[10]



Scheme 1 Plasma polymer encapsulation of ampicillin for sustained release using a plasma reactor with an additional platform to induce continuous agitation of powdered substances within the plasma field.

ChemComm Accepted Manuscript

To control the rate of release of ampicillin we chose to vary the thickness of the capsule wall. Fig. 1a shows the polymer film thickness, determined by ellipsometry, as a function of time when deposited on planar surfaces. The film thickness increases linearly in the studied time of 20 minutes with a rate of 28 nm per minute.

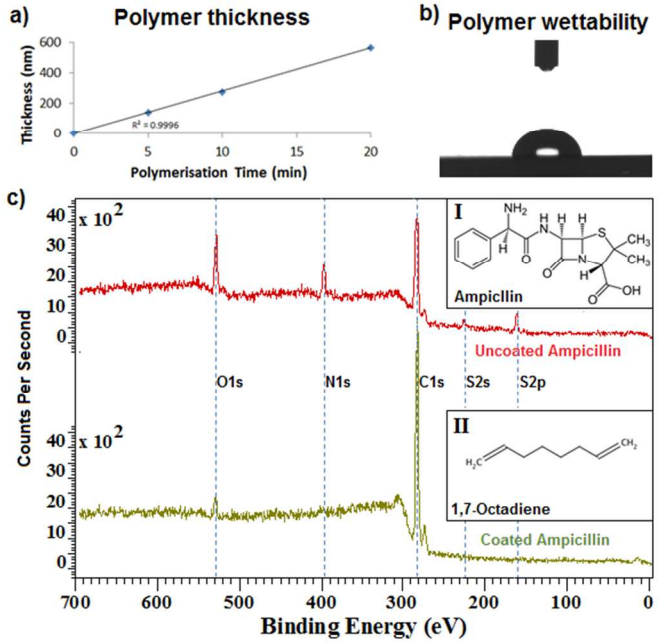


Figure 1 (a) Thickness of plasma deposited 1,7-octadiene layer on planar samples for various time periods. (b) Water contact angle of plasma polymer coating deposited from 1,7-octadiene. (c) XPS spectrum of uncoated ampicillin (top) and after encapsulated with ppOD for 5 minutes (bottom), and the corresponding to chemical structures of ampicillin (inset I) and 1,7-octadiene precursor (inset II).

When deposited on particles, the thickness of the coating would be lower compared to that deposited on a planar surface for the same time. This is intuitive to understand taking into account the high surface area of the particles compared to planar surface. However, the principle of increased coating thickness with longer deposition time would still hold. This gives us the capacity to control the thickness of the capsule outer wall. The plasma polymer coatings deposited from 1,7-octadiene had advancing water contact angles of 81° ±2°, image shown in Fig. 1b which is consistent with published studies.[11]

Ampicillin powder (as received) was added to the plasma chamber as received and coated whist being continuously agitated (details of the samples holder are given on Fig. S1). The chemical composition of the powders was determined via XPS before and after coating. Fig. 1c shows a clear difference in the intensity of the N1s and S2p peaks, at 399.5 eV and 163.0 eV, respectively, between the uncoated ampicillin (top spectrum) and the 1,7-octaidene encapsulated ampicillin (bottom spectrum). As seen in the chemical structure of ampicillin, the molecule contains nitrogen and sulphur which give clear peaks in the XPS survey spectrum. After coating for 5 minutes nitrogen and sulphur can no longer be seen. This suggests that the coating (1,7-octaidene does not contain nitrogen or sulphur) of the outer surface of the powder is uniform and thicker than the sampling depth of XPS (ca 10 nm). Three different coating times were used to establish different thicknesses of the outer shell: 5, 10 and 20 minutes (A05, A10 and A20 respectively).

Fig. 2 shows the release profile of the encapsulated ampicillin. The concentration of dissolved drug in aqueous medium over time was determined by UV-Vis spectroscopy using the intensity of the absorbance band at 268 nm.

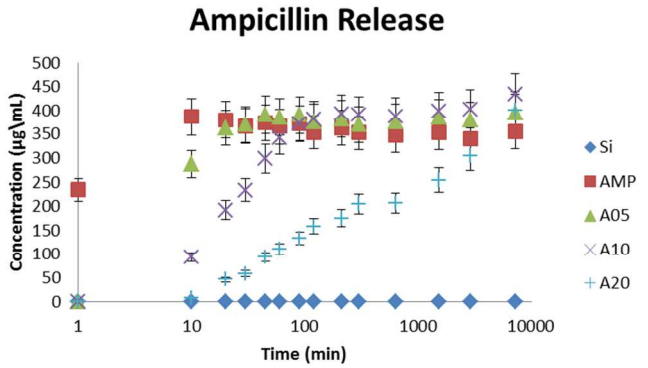


Figure 2 Ampicillin release rates over 5 day period. Where release of ampicillin into MIlli-Q water from coated and uncoated ampicillin powder based on UV-Vis absorbance band (268nm) where AMP (■), A05 (▲), A10 (x), and A20 (+) (uncoated ampicillin and ampicillin with 5, 10 and 20 minute ppOD coatings respectively) showed different release rates depending on polymerisation time. Si powder coated with ppOD (♦) was used as a control. All error assumes a ±10% deviation.

Uncoated ampicillin was fully dissolved within 10 minutes. Powders coated for 5 and 10 minutes (A05 and A10) released all their contents within 20 and 90 minutes, respectively (Figure S2). The sample coated for 20 minutes (A20) showed sustained release of the ampicillin for 5 days. Si powder coated with ppOD was used as an ampicillin-free control. There is a clear correlation between the time taken to fully dissolve the coated ampicillin and the plasma polymer deposition time used for encapsulation. This indicates that the capsule thickness plays a crucial role in regulating ampicillin release through restricting the ability of water to penetrate the ppOD coating and dissolve the ampicillin, and also the capacity of the dissolved ampicillin to pass through the coating. It is interesting to note that capsules built for 5 and 10 minutes were not capable of significantly reducing the release rate of the drug. This suggests that the thickness of the coating may not have been sufficient to completely encapsulate the drug particles. Although the particles were continuously agitated to provide uniform coating, the thickness of the capsule wall (few tens of nanometers) is very small relative to the size of the particles (few tens to few hundreds of microns as seem from microscopy images in Fig. S3). In addition, particle shape is not spherical. Thus, irregularities in particle shape, such as edges and groves (Fig. S3), may not have been fully coated allowing direct exposure to the solvent when deposition times were 5 and 10 minutes. These issues were fully overcome when the deposition time was 20 minutes. This shows the capacity of the technology to provide encapsulation of particles of complex shapes and diversity of sizes. However, it also shows that the coating

ChemComm Accepted Manuscript

This journal is © The Royal Society of Chemistry 2012

thickness needs to be optimised for a particular pharmaceutical based of the size and shape of the respective particles.

Importantly for biological applications, we have conducted comparative release studies of the A20 sample in PBS. The results are shown in Figure S4 of the supplementary information demonstrate almost identical release kinetics. To demonstrate that the technology is applicable to other drugs we coated vancomycin particles (as supplied) with ppOD for 20 minutes. However, since the vancomycin particles size distribution is different to that of ampicillin (Figure S5), the resultant release profile follows a different kinetic (Figure S6). This demonstrates that that the condition of encapsulation need be optimised for every individual drug. Future studies in our lab will aim at establishing (if possible) a universal relationship between drug particle size distribution and capsule thickness and material.

Ultimately, it is important to demonstrate that in addition to a sustained and controlled release the drug is in active form after release. Table 1 shows the MIC values to *Staphylococcus epidermidis* and *Escherichia coli* obtained using the dissolved ampicillin with and without encapsulation. The values for *S. epidermidis* and *E. coli* were 50 and 3.125 µg\mL respectively. There are no differences in the MIC values between the coated and the uncoated ampicillin. This result suggests that the plasma deposition process did not affect the activity of the drug. The MIC values and the release kinetics shown in Fig. 2 also demonstrate that at any point of time the drug concentration is above the MIC values. The effect of the capsule material on the MIC value was also investigated. The data shown in Table S1 shows negligible effect on *E. coli* and no *S. epidermidis* when tested against coated silica powder.

Sample	*S. epidermidis* MIC (µg\mL)	*E. coli* MIC (µg\mL)
AMP	50	3.125
A05	50	3.125
A10	50	3.125
A20	50	3.125

Table 1 MIC values obtained from leached ampicillin from neat ampicillin and ppOD encapsulated ampicillin (A05, A10 and A20) for *S. epidermidis* and *E. Coli* showing no change in efficacy.

In summary, we have developed a novel solvent-free encapsulation technology which can be used to control the rate of drug release. We demonstrate the feasibility of the technology with ampicillin as a model drug. We achieved sustained release over 5 days and provided evidence that the encapsulation process does not alter the activity of the compound. The technology presented in this communication has an enormous potential for application with variety of drugs and medical treatments. Considering the vast variety of plasma polymerizable precursors, the physical and chemical properties of the outer capsule, as well as the wall thickness, are easily customizable and could be fine-tuned to suit different applications. Herein, we demonstrate the controlled release of hydrophilic drugs, however, we hypothesize that utilising the same technology but involving hydrophilic coatings can be used to enhance the dissolution and bioavailability for poorly-soluble drugs. The capsules can also be prepared using functional plasma polymers and further decorated with specific ligands that would open enormous opportunities into the field of targeted drug delivery for treating diseases such as cancer.

Acknowledgements

KV thanks to ARC for fellowship No FT100100292.

Notes and references

[a] School of Engineering, University of South Australia, Mawson Lakes, Adelaide, SA 5095, Australia.
[b] Mawson Institute, University of South Australia, Mawson Lakes, Adelaide, South Australia 5095, Australia. E-mail: krasimir.vasilev@unisa.edu.au

†*Experimental:* A bell chamber plasma reactor with an additional port for DC current input was used for all plasma reactions. Dry powders were coated by using a shaking platform comprised of a centre motor with DC voltage speed dependence and an offset weight to induce a random shaking motion (Fig. S2). Ampicillin powder was used as purchased, brought to room temperature and placed on a Petri-dish attached to the platform, without any prior modification to the powder. The chamber was brought to a base pressure of $3x10^{-2}$ mbar and 1,7-octadiene flowed into the chamber until a steady pressure of $2.1x10^{-1}$ mbar was obtained. Plasma was induced using a 50W 13.56MHz radio frequency for various times whist the shaking platform was operating: providing continuous agitation to the particles. After deposition 1,7-octadiene flow was stopped and the chamber was returned to base pressure to remove any unreacted precursor. The chamber was vented and Perti-dish containing coated ampicillin removed.

Coated ampicillin (5mg) was immersed in MilliQ filtered water to a precise concentration of 400µg\mL and placed on a mechanical shaker for predetermined time periods of time. Absorbance was measured via UV-Vis spectroscopy (268nm) to determine the concentration of the leached ampicillin. A calibration curve was constructed by measuring the absorbance of neat ampicillin dissolved in MilliQ filtered water at concentrations of 1000µg\mL to 15.625 µg\mL. ppOD coated silica was used as a control.

Ampicillin efficacy was determined by looking at any changes in MIC against *S. epidermidis* (ATCC 35984) and *E. coli* (ATCC 25922) post coating with ppOD. The encapsulated ampicillin was allowed to leach for 24h in MilliQ filtered water before MIC was performed. 25µL of serial diluted leached ampicillin solution in filtered water was brought to a final working volume of 200µL with TSB and an end concentration of $1x10^6$ CFU\mL bacteria. The MIC was determined by looking at wells with no turbidity. Results were confirmed by measuring optical density at 600nm using a Biotek ELx800.

Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/c000000x/

1(a) Y. Zhang, H. F. Chan and K. W. Leong, *Advanced Drug Delivery Reviews*, 2013, **65**, 104; (b) K. E. Uhrich, S. M.

ChemComm Accepted Manuscript

Cannizzaro, R. S. Langer and K. M. Shakesheff, *Chemical Reviews*, 1999, **99**, 3181; (c) T. M. Allen and P. R. Cullis, *Science*, 2004, **303**, 1818.

2(a) J.-C. Pechère, D. Hughes, P. Kardas and G. Cornaglia, *International Journal of Antimicrobial Agents*, 2007, **29**, 245; (b) T. F. Blaschke, L. Osterberg, B. Vrijens and J. Urquhart, *Annu. Rev. Pharmacol. Toxicol.*, 2012, **52**, 275.

3(a) K. Langowska, J. Kowal, C. G. Palivan and W. Meier, *Journal of Materials Chemistry B*, 2014, **2**, 4684; (b) B. Jeong, Y. H. Bae, D. S. Lee and S. W. Kim, *Nature*, 1997, **388**, 860; (c) P. Yang, S. Gai and J. Lin, *Chemical Society Reviews*, 2012, **41**, 3679.

4(a) J. Naik, A. Lokhande, S. Mishra and R. Kulkarni, *Int J Pharm Bio Sci*, 2012, **3**, 573; (b) M. M. de Villiers and Y. M. Lvov, *Advanced Drug Delivery Reviews*, 2011, **63**, 699.

5(a) G. Baier, A. Cavallaro, K. Vasilev, V. Mailänder, A. Musyanovych and K. Landfester, *Biomacromolecules*, 2013, **14**, 1103; (b) S. K. Yandrapu, A. K. Upadhyay, J. M. Petrash and U. B. Kompella, *Molecular Pharmaceutics*, 2013, **10**, 4676; (c) P. W. Labuschagne, R. Adami, S. Liparoti, S. Naidoo, H. Swai and E. Reverchon, *The Journal of Supercritical Fluids*, 2014, **95**, 106; (d) C. Celia, S. Ferrati, S. Bansal, A. L. van de Ven, B. Ruozi, E. Zabre, S. Hosali, D. Paolino, M. G. Sarpietro, D. Fine, M. Fresta, M. Ferrari and A. Grattoni, *Advanced Healthcare Materials*, 2014, **3**, 153.

6 World Health Organization, *The world health report*, 2004.

7 K. Vasilev, N. Poulter, P. Martinek and H. J. Griesser, *ACS Applied Materials & Interfaces*, 2011, **3**, 4831.

8 S. Simovic, D. Losic and K. Vasilev, *Chemical Communications*, 2010, **46**, 1317.

9 I. Braceras, J. Oyarbide, P. Azpiroz, N. Briz, E. Ipiñazar, N. Álvarez, G. Atorrasagasti, R. M. Fratila and J. M. Aizpurua, *Plasma Processes and Polymers*, 2013, **10**, 328.

10 X. Liu, Q. Feng, A. Bachhuka and K. Vasilev, *Applied Surface Science*, 2013, **270**, 473.

11(a) K. Vasilev, A. Mierczynska, A. L. Hook, J. Chan, N. H. Voelcker and R. D. Short, *Biomaterials*, 2010, **31**, 392; (b) A. Michelmore, A. Mierczynska, Z. Poh, R. V. Goreham, D. Losic, R. D. Short and K. Vasilev, *Nanotechnology*, 2011, **22**, 415601. (c) B. Akhavan, K. Jarvis and P. Majewski, *Plasma Processes and Polymers*, 2013, **10**, 1018;

ChemComm Accepted Manuscript

 This journal is © The Royal Society of Chemistry 2012