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Research paper

Characterisation of human saliva as a platform for oral dissolution medium development

Sally Gittings [a], Neil Turnbull [b], Brian Henry [b], Clive J. Roberts [a], Pavel Gershkovich [a,*]

[a] School of Pharmacy, The University of Nottingham, Nottingham, United Kingdom
[b] Pfizer Drug Product Design, WRD Pharmaceutical Sciences, United Kingdom

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ABSTRACT

Human saliva is a biological fluid of great importance in the field of dissolution testing. However, until now, no consensus has been reached on its key characteristics relevant to dissolution testing. As a result, it is difficult to select or develop an *in vitro* dissolution medium to best represent human saliva. In this study, the pH, buffer capacity, surface tension, viscosity and flow rate of both unstimulated (US) and stimulated (SS) human saliva were investigated in order to provide a platform of reference for future dissolution studies using simulated salivary fluids. Age and gender related differences in a sample size of 30 participants for each parameter were investigated. Significant differences were established between US and SS for all characteristics except surface tension. Therefore, the requirement for using two simulated salivary fluids should be considered when developing an oral dissolution model.

1. Introduction

The oral cavity as a dissolution site is often overlooked due to rapid oral transit as conventional dosage forms are swallowed. However conventional oral formulations such as tablets and capsules are of limited application in the paediatric and geriatric population and alternative oral dosage forms which reside in the mouth for a significant time are increasing in popularity [1]. In addition, adult dosage forms which can be taken "on the move", without the co-administration of water are also gaining interest [2]. Many alternative formulations, such as oral films, sublingual and buccal tablets and orally disintegrating tablets rely on dissolution or disintegration in saliva. On the contrary, taste masked oral dosage forms often aim to reduce drug dissolution in saliva in order to prevent contact between the unpleasant tasting active pharmaceutical ingredient (API) and the taste buds [3]. Saliva therefore plays a critical role in the dissolution and performance of these formulations. However, there is no consensus on the key characteristics of human saliva which may affect dissolution, and as a result, it is difficult to select or develop an *in vitro* dissolution medium to best represent human saliva in the evaluation of these dosage forms.

A number of parameters can be considered as highly influential on *in vitro* dissolution. The pH, buffer capacity and surface tension are identified as some of the most important factors [4]. Additionally, viscosity is considered in many cases [5]. Furthermore, Wang et al. described biorelevant dissolution and suggested consideration of pH, buffer capacity, surface tension and viscosity of the medium to be paramount for biorelevant dissolution testing (along with non-medium related hydrodynamic factors such as volume, flow, agitation and apparatus) [6]. The importance of these particular parameters is evident as similar approaches have been adopted in the characterisation of other gastro-intestinal fluids [7–13]. Extensive research has been carried out over the past few decades in the design and development of, and application of, biorelevant media representing other gastro-intestinal fluids in both the fed and fasted states [11,14–20]. However, saliva remains less well characterised. Without knowledge of these crucial medium parameters in human saliva, it would prove impossible to develop or select a biorelevant simulated salivary fluid for dissolution studies. This paper therefore aims to address the gap in the characterisation of human biological fluids through the investigation of key parameters of saliva.

The pH of a dissolution medium is important since it affects ionisation of the API, according to the Henderson–Hasselbalch equation, and ionisation is directly linked with the aqueous solubility

Abbreviations: API, active pharmaceutical ingredient; SS, stimulated saliva; SSF, simulated salivary fluid; US, unstimulated saliva.

* Corresponding author. Centre for Biomolecular Sciences, School of Pharmacy, University Park, University of Nottingham, Nottingham NG7 2RD, United Kingdom. Tel.: +44 0115 84 68014.
E-mail address: pavel.gershkovich@nottingham.ac.uk (P. Gershkovich).

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of an API [21]. Of equal importance therefore is the ability of the medium to resist changes in pH as an acidic or basic drug begins to dissolve, i.e. the medium's buffer capacity. This was demonstrated by Tsume et al. [22] who performed dissolution experiments in media of different buffer capacities with the acidic drug ibuprofen and found that when the buffer capacity was low, the pH decreased to a greater extent as dissolution proceeded, which hindered the rate and extent of further dissolution.

The pH of human saliva has been described previously, with varying results in the wide range of 5.3–7.8, depending on the stimulation state [10,23]. In most studies, either unstimulated saliva (US) or stimulated saliva (SS) was investigated, but not both [24,25]. Additionally, studies in which the pH of both types of saliva was investigated generally had a small number of participants, or focussed on just one or two salivary characteristics relevant to dissolution testing, such as Bardow et al. [26] who investigated only the pH and buffer capacity. We aim to address this issue by characterising the pH of both US and SS, as well as other key parameters within the same sample.

Buffer capacity has been investigated in numerous studies. However, in most cases, the experimental design employed does not allow one to draw conclusions about the actual buffer capacity value or range. Literature values are reported in different ways. Some research groups have simply quoted the buffer capacity to be high, medium or low, without providing any actual value [27]. Thus one cannot draw direct comparisons between studies. Some researchers simply state the bicarbonate concentration of saliva samples to infer buffer capacity [28]. Furthermore, in some cases, buffer capacity is quoted in mmol L^{-1} pH^{-1} [26,29]. The lack of similarity in experimental design has led to inconclusive findings regarding the buffer capacity of saliva. This research aims to address these issues by assessing the buffer capacity of saliva using similar experimental design to that used for other gastro-intestinal fluids [12,13,16,20] to allow for comparison.

Viscosity is another key parameter affecting dissolution. A high viscosity medium would increase the thickness of the boundary layers (h) and decrease the diffusion coefficient (D) according to the Noyes–Witney dissolution model, thus reducing the drug dissolution rate compared with a medium of lower viscosity [30]. Despite viscosity of stimulated and unstimulated whole human saliva being evaluated by several research groups, no consensus has been reached on human saliva viscosity due to differences in experimental conditions. For example, in a review by Schipper et al. [31] viscosity of unstimulated whole saliva was found to be 1.5–1.6 mPa s over a shear rate of 1–300 s^{-1} in one study [32]. However another study found it to range from 3.8 to 8.8 mPa s at a single shear rate of 90 s^{-1} [33] and a viscosity of 100 mPa s was recorded at a shear rate of 0.02 s^{-1} in another study [34] within this review. Research groups used different shear rates, temperatures and types of rheometer and often small sample sizes. This research aims to address these issues by using physiological temperature and assessing viscosity across a wide range of shear rates.

It is well known that the surface tension of the medium also affects the rate of dissolution [9]. A high interfacial tension reduces wetting of the drug particles and reduces the rate of dissolution. Wetting can be improved by the addition of surfactants, reducing interfacial tension and increasing the rate of dissolution, and it is a common practice to add surfactants to dissolution media [35]. Although many studies have investigated the film forming properties of saliva, as well as salivary pellicle thickness and composition [36], few studies have focussed on the surface tension of whole human saliva [23]. Literature regarding the surface tension of saliva uses variable experimental designs including different temperatures and sites in the oral cavity, and often small or non-specified numbers of participants [37,38]. Further clarification of this parameter is therefore required, using a sufficient number of samples and physiologically relevant temperature.

Despite not directly affecting media choice and composition, salivary flow rate is an important factor when developing a biorelevant dissolution model [6]. The volume available for dissolution, or flow rate, should reflect physiological conditions since this affects the concentration gradient of solvated API molecules and saturation of the bulk fluid. Salivary flow rate has been investigated; however, most groups investigated either US [24] or SS [39]. Since inter-individual variation is so vast in these studies, flow rate should be considered for US and SS in the same individual to allow accurate comparison of stimulation states.

Many research groups performing dissolution tests modelling the oral environment have not taken into account the key characteristics described above and have used simple media such as water or phosphate buffered saline (PBS) [3,40]. The most likely reason for that is the lack of availability of sufficient data on the characteristics of human saliva. In addition, many commercial [41] and literature formulations [42] for simulated salivary fluids (SSF) exist. In fact, one article in the year 2000 identified 60 artificial salivas of differing compositions. Whilst it is possible that some of these 60 compositions may be appropriate for dissolution studies, information regarding parameters key to dissolution testing is not available [43]. It is also difficult to select the single most appropriate SSF without a clear understanding of human salivary characteristics, and therefore these SSFs are rarely used in dissolution testing. Saliva is a complex mixture containing 99% water, but additionally contains numerous electrolytes, small organic molecules such as hormones and glucose, and proteins such as immunoglobulins, enzymes and glycoproteins, which may have a huge impact on dissolution [44].

Therefore, the aim of this work was to characterise stimulated and unstimulated human saliva for the key characteristics relevant to dissolution to provide a platform of reference for the future selection or development of oral dissolution media that would be representative of human saliva. The saliva flow rate was assessed in this work to aid development of oral dissolution models. Age and gender related differences were also investigated for each parameter. To the best of our knowledge, this is a first work in which the key parameters relevant to drug dissolution – pH, buffer capacity, viscosity, surface tension and flow rate – are assessed simultaneously for both stimulated and unstimulated whole human saliva with a sufficient number of participants to draw statistically meaningful conclusions.

2. Methods and instrumentation

2.1. Human volunteers

All saliva samples were collected in accordance with ethical approval number R12122013 SoP TTTFS, Faculty of Medicine and Health Sciences Research Ethics Committee, Queens Medical Centre, Nottingham University Hospitals. Participation was voluntary and informed written consent was obtained. All data were held in accordance with the Data Protection Act.

Participants were recruited from the University of Nottingham and were healthy adult volunteers. Exclusion criteria included chronic or acute illness in the past 3 months, cold or flu symptoms, oral health concerns and any medication, with the exception of contraception. Participants were asked not to eat, drink, smoke or use oral hygiene for 2 h prior to donation. Donations took place at approximately 3 pm in the afternoon to avoid diurnal salivary changes. The study group demographics are shown in Table 1. The study group was mostly Caucasian (26 of 30 participants).