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Research paper

Use of dye as tracer of drug release from medicated chewing gums

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Abstract

The evaluation of the potential use of a dye as indicator of *in vivo* drug release from a medicated chewing gum is described. The device is a three-layer tablet obtained by direct compression consisting of a gum core and two external protective soluble layers to prevent gum adhesion to the punches of the tableting machine. The active ingredient and a colour are contained in the gum core. To evaluate the drug and the dye release from the formulations, a chew-out study was performed by a panel of volunteers. The results obtained suggest that the use of a dye could be useful to indicate the chewing time necessary to complete drug delivery from medicated chewing gums.

Keywords: Medicated chewing gum; Three-layer tablet; Dye; Chew-out study; *In vivo* drug release

1. Introduction

The European Pharmacopoeia defines medicated chewing gums as "solid, single-dose preparations with a base consisting mainly of gum that are intended to be chewed but not swallowed" [1]. They can be used therapeutically for local treatment of buccal mucosa diseases or for systemic treatment after drug absorption.

The release rate of the active, from a chewing gum, is determined by the physico-chemical characteristics of the drug, by the composition and manufacturing process of the formulation, and by the patient chewing performances [2,3]. People show different chewing time, chewing frequency and chewing intensity, moreover patients with xerostomia or oromucosal diseases may experience chewing difficulties altering their chewing performances [4,5]. For all these reasons the drug released from a chewing gum may show large inter-individual variations.

In earlier studies, the experimental protocols carried out to evaluate the *in vivo* drug release rate from medicated chewing gums were designed fixing chewing time and chewing frequency in order to minimize inter-individual variations [6–9].

In 1991, Barabolack et al. published a study where the average chewing time (in minutes) was determined; the results were based on more than 4000 American volunteers. The mean chewing time found was 36 min. On the basis of their results, the authors suggested that a chewing time of 30 min could be targeted for the design of a clinical trial. However, a chewing time of 20 min can be applicable to more than 80% of the gum chewers participating in the study [10].

The approach of our study was to design a chewing gum where the complete release of the drug dose from the formulation can be detected from an organoleptic change of the gum (in this case, the loss of a colour) independently of the different chewing times and chewing frequency of the patients.

In this work, medicated chewing gums were formulated containing a proper amount and type of dye, used as indicator of the drug release process from the device. Drug and

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colour are released at the same time while the patient chews the gum; the loss of the gum colour could be an indicator of complete drug release. To obtain this target, it is necessary that the colour would be released at the same rate of the drug or at least slightly slower, in this way we can be sure that the whole dose of active has been delivered when the gum loses all, or almost all, its original colour.

Specifications do not exist regarding the limits of drug release from medicated chewing gum in any Pharmacopoeia; for this reason, in this work, it has been established that the optimal formulation should be able to release more than 90% of the whole dose of the active before the chewed-gum becomes completely colourless and within 30 min of chewing.

The chewing gums proposed are three-layer tablets in which a gum core is protected by two external layers for industrial production feasibility. The gum core is composed of a gum base, which is a mixture of substances able to yield a suitable texture to the chewing gum (softness/hardness, elasticity, plasticity, stickiness, crumbliness). The core also contains the active and the colour doses. Additional sweeteners and flavouring agents were previously selected (unpublished data) and added to the core to mask the drug taste and allow the patient to chew the product for the needed time. The protective external layers were designed to prevent tablet adhesion to the punches of the tableting machine, during direct compression; thus, a combination of excipients and lubricants were selected for this purpose, furthermore, flavouring agents and sweeteners were used to improve the organoleptic characteristics of the tablet. For the preparation of this kind of device, a novel technology previously described and validated is used [11]. In this manufacturing process, the tablets are prepared at room temperature by direct compression of the materials. The main advantage of this technology consists in the use of conventional pharmaceutical equipment, like multi-layer tableting machines. Furthermore, avoiding the high temperatures used by traditional methods of chewing gum preparation, it is possible to use thermolabile substances as well as to improve the physical and chemical stability of the whole formulation.

Metoclopramide Hydrochloride (MCP), Ascorbic Acid (AAC) and Caffeine (CAF) can be formulated in chewing gums, for this reason they were chosen as model drugs for this study.

Traditionally, colours are included in pharmaceutical products to increase the patient acceptability and producing an eye-appealing product, they have also been used to mask or modify undesirable appearance of the pharmaceutical preparation, furthermore, colours are used to distinguish between two or more different products. In this study, the colours are not used as additive to improve organoleptic characteristics of the product, but they work as tracers of drug release from chewing gum formulations. The colours chosen were: Quinoline yellow (E104) and Sunset Yellow (E110). They are water soluble dyes, approved by EU directives [12], used in food and pharmaceutical products. Previous experiments carried out in our laboratory (unpublished data) demonstrated that both dyes are good candidates to be used in chewing gum formulations because they do not spot the teeth while the patient chews the gum and because, from a technological view point, they yield a homogeneous coloured aspect to the gum.

2. Materials and methods

2.1. Materials

Metoclopramide (MCP) (AMSA, Como, Italy), Ascorbic Acid (AAC) (Roche, Basel, CH) and Caffeine (CAF) (Carlo Erba, Milan, Italy) were used in the dose of 10, 50 and 50 mg, respectively.

Sunset Yellow (Dye6) and Quinoline Yellow (Dye10) were supplied by Colorcon Limited (Kent, UK).

The gum base used for the preparation of the devices was obtained from Gum Base Company S.p.A. (Lainate, Italy); flavouring agents were supplied by Officina degli Aromi S.r.l (Morazzone, I) and sweeteners from Sigmar Italia (Almè, I).

The other excipients used were: Maltodextrin (Glucidex IT19®, Roquette, Lestrem, France), Magnesium stearate, Talc (Carlo Erba, Milan, Italy), Colloidal Silicon oxide (Syloid 244, Grace GmbH, Worms, D).

For the quantitative analysis, ammonium acetate, and metaphosphoric acid were analytical grade reagents supplied by Carlo Erba (Milan, I). Methanol and Acetonitrile HPLC grade were used (Carlo Erba, Milan, Italy). HPLC quality water was produced with a Milli-Q® water purification system (Millipore, Bellerica, USA).

3. Methods

3.1. Tablets preparation

The compositions of the prepared formulations are reported in Table 1.

Table 1
Composition (mg) of the core and external layers of the chewing gum formulations

Formulation	MCP1	MCP3	MCP5	AAC3	CAF3
Inner core					
Drug	10	10	10	50	50
Dye6	1	3	5	3	–
Dye10	–	–	–	–	3
Gum base	900	900	900	1000	900
Flavours and sweeteners	120	120	120	24	40
Lubricants	4.5	4.5	4.5	8	10
External layer					
Maltodextrin	300	300	300	300	300
Flavours and sweeteners	5	5	5	4	4
Lubricants	9	9	9	18	18

For the formulations containing Caffeine (CAF3) and Ascorbic Acid (ACC3) the dye dose used was 3 mg. Instead, three Metoclopramide formulations (MCP1, MCP3 and MCP5) were prepared with three different dye content (1, 3 and 5 mg) to select the proper amount needed to obtain comparable dye and drug releases.

The three-layer tablets were prepared using a reciprocating tablet machine (Korsh EKO, Berlin, D) equipped with 14-mm diameter flat punches. The die of the machine was manually filled with the weighed amounts of the lower layer, of the core formulation and of the upper layer and compression cycle was activated.

3.2. Drug and dye release evaluation

An *in vivo* chew-out study was performed to evaluate drug and dye release from the chewing gums described. The formulations were chewed by a panel of three healthy volunteers. The subjects were asked not to eat or drink or smoke while chewing. Each formulation was chewed for given periods of time (10, 20, 30 min). For the drug and dye assessment, the original tablets and/or their residual chewed-gum were previously frozen and ground. The powder obtained and accurately weighed was transferred into a volumetric flask and diluted with a specific extraction solution then, the samples were sonicated and stirred for 15 min and 120 min, respectively.

A Variant HPLC system, equipped with a Polaris 5 μ C18-A column, was used for the chromatographic assays of MCP, AAC and Dye6 (Solvent Delivery System model 9010, Variable Wavelength UV–vis detector model 9050).

For CAF and Dye10 determination, a direct UV-method was used, it means that the samples prepared as above (extraction solution: water) were filtered and analyzed directly with UV–vis spectrophotometer at λ 273 nm for CAF and 501 nm for Dye10 (Spectracomp 602, Advanced products, Milan, Italy).

The difference between the initial dose of the active and its residual amount found in the chewed-gum corresponds to the amount of substance delivered at each chewing time.

Placebo formulations (corresponding to MCP1, MCP3, MCP5 and CAF3), containing the dye doses studied, were also prepared and chewed by a panel of three volunteers (three replicates) to evaluate the colour intensity of the corresponding chewed cuds at the previously fixed time: 10, 20 and 30 min. The residual gums were moulded in a suitable die and then photographed using a digital microscope camera (SV Micro™, Sound Vision Inc., MA, USA).

4. Results

A comparison between the physical aspect (see photos in Figs. 1 and 2) and the quantitative analysis of dye contained in the residual gums was performed to establish the amount of dye needed to consider the gum colourless. In the case of the formulation containing Dye10 (CAF3) this amount is 0.02 mg, and corresponds to the photo of the gum chewed for 30 min (Fig. 1); it means that we consider that the residual cud, from CAF3 formulation, is colourless when the amount of Dye10 remained in it is less than 0.02 mg. For the formulations containing the Dye6 (MCP1, MCP3, MCP5) this amount is about 0.06 mg and the colour observed corresponds to Fig. 2(b_1).

To evaluate the drug released from the chewing gums, their residuals after 10, 20 and 30 min of chewing were analyzed; the difference between the initial dose of active present in the tablet and the amount of active found in the residual cud represents the amount of drug released for each time. The percentage of active released and the amounts of residual dye found in the chewed-gums were plotted vs. chewing time.

An immediate confirmation of how the formulations work is shown in Fig. 3, where the percentage of MCP released and the amount of residual dye from MCP3 formulation after 10 min of chewing is shown for each volunteer. While for volunteer A more than 90% of MCP is released and the residual dye is less than 0.1 mg (in fact the cud is almost colourless), at the same time, 10 min, the gums chewed by volunteers B and C have delivered about 80% of the drug and the residuals are still coloured, as indicated by the dye content found in the chewed gum (around 0.3 mg). This result shows how the chewing performances of the individuals can influence both drug and dye release from this type of formulation and confirms that the dye is able to indicate the drug release efficiency for each volunteer.

The results (mean of volunteers) obtained from the chewing gum formulation containing Caffeine (CAF3) are

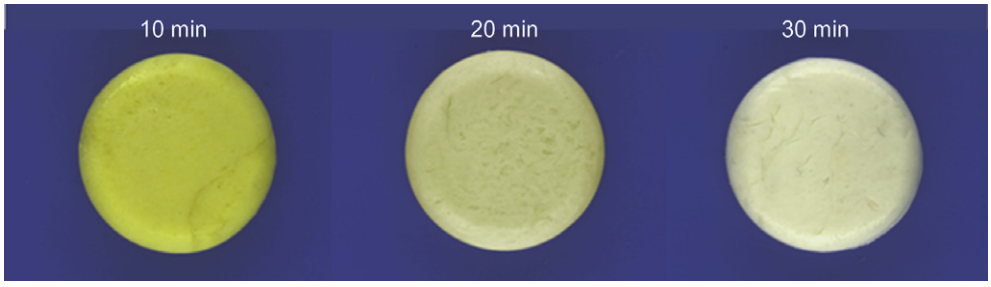

Fig. 1. Photos of the residual gums obtained after: 10, 20 and 30 min of chewing from placebo chewing gums containing 3 mg of Dye10 (mean of volunteers).

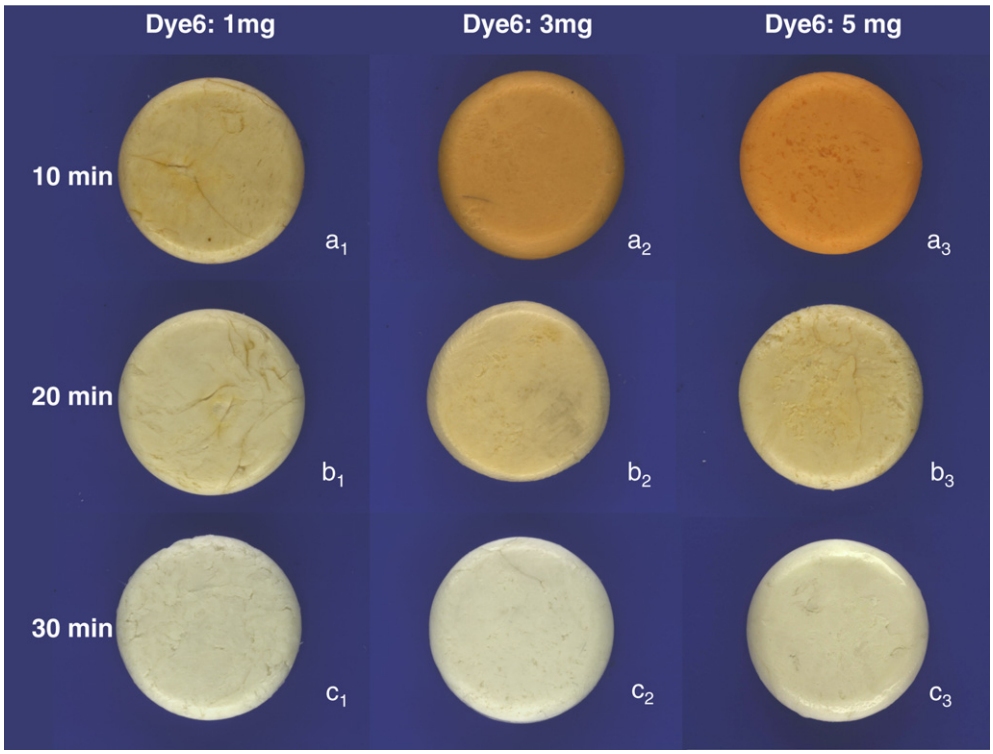

Fig. 2. Photos of the residual gums obtained after: 10, 20 and 30 min of chewing, from placebo chewing gums containing 1, 3 and 5 mg of Dye6 (mean of volunteers).

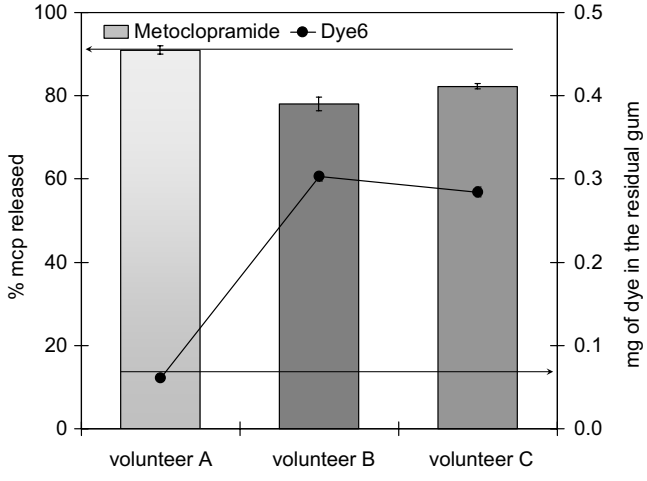

Fig. 3. Percentages of MCP released (left scale) and mg of Dye6 (right scale) contained in the residual gum from MCP3 after 10 min of chewing for each volunteer.

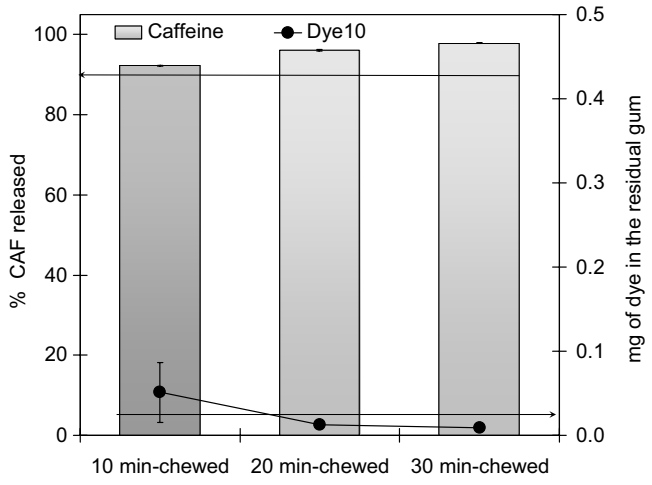

Fig. 4. Comparison between % of Caffeine released (left scale) and the mg of Dye10 (right scale) remained in the chewed-gum from CAF3 formulation (mean of volunteers).

shown in Fig. 4. After 10 min of chewing, more than 90% of CAF dose is delivered, and the residual gum is still yellowish (0.05 mg of Dye10 found in the cud). Increasing the chewing time to 20 min, an increase in drug release is obtained, in fact, Caffeine delivery is over 95%, and the amount of dye decreases to 0.01mg, the residual cud obtained at this time point is less coloured compared to the 10 min-chewed gum (Fig. 1). After 30 min of chewing, the residual gum is colourless (0.009 mg of dye found in the cud) and the amount of drug delivered is practically invariable compared to the value found at 20 min. On the basis of these results it is possible to conclude that this formulation (CAF3) is able to release more than 90% of the whole dose, before the gum becomes colourless (in about 20–30 min).

For the Ascorbic Acid formulation (ACC3) the percentage of active released after 10 min of chewing is 95% (Fig. 5), and the amount of residual dye in the gum is more

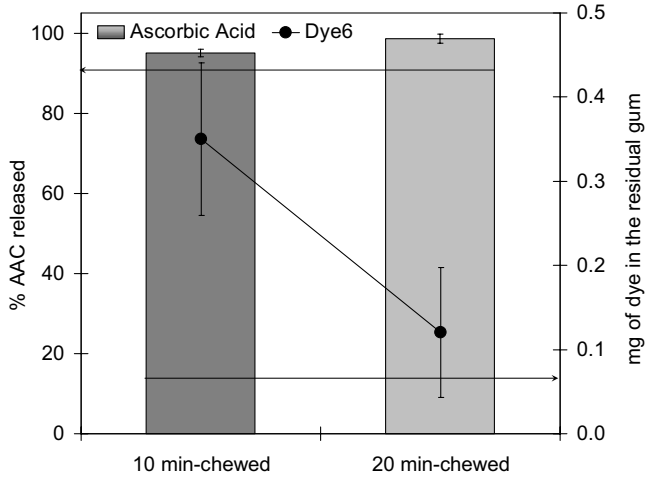

Fig. 5. Comparison between % of Ascorbic Acid released (left scale) and the mg of Dye6 (right scale) remained in the chewed-gum from AAC3 formulation (mean of volunteers).

than 0.3 mg. Increasing chewing time to 20 min, the whole dose of Ascorbic Acid is delivered, and the residual gum is almost colourless containing only 0.1 mg of Dye6. No determination at 30 min of chewing was performed because after 20 min the formulation releases all the AAC dose and the chewed gum is almost colourless, this result indicates that a dose of 3 mg of Dye6 is suitable to indicate the whole release of AAC from the formulation.

The percentage of MCP released from MCP1, MCP3 and MCP5 formulations (dye dose: 1, 3 and 5 mg, respectively) after 10 min of chewing ranges between 73% and 85% (Fig. 6). In the 10 min-chewed gum, obtained from MCP1, less than 0.1 mg of dye is still present, in fact, the residual gum is almost colourless (Fig. 2a_1). When MCP3 and MCP5 formulations were chewed for 10 min, higher amounts of dye (about 0.2 mg) were found in their residuals compared to MCP1 formulation (Fig. 6), in fact, the photos corresponding to MCP3 and MCP5 cuds showed intense orange coloration (Fig. 2a_2, a_3). All the values assessments, either percentage of MCP released or amount of residual dye, in the gums chewed for 10 min, showed high standard deviations, this means that a high inter-individual variation among the volunteers does exist and suggests that drug and dye released at this time (10 min) strongly depends on chewing performances of the individual.

As expected, by increasing chewing time to 20 min (Fig. 7), both drug and dye release increases: the percentages of MCP delivered from the three formulations (MCP1, MCP3 and MCP5) are quite similar (about 90%). The amount of dye found in the 20 min-chewed gums, obtained from MCP1 and MCP3, is less than 0.06 mg, the photos corresponding to these residuals are almost colourless. On the contrary, MCP5 formulation produces residual gums slightly coloured, compared to MCP1 and MCP3 residual cuds, at the same time point (Fig. 2b_1, 2b_2 and 2b_3).

By increasing the chewing time to 30 min, the percentage of MCP released is about 93% for the three formulations (Fig. 8); no trace of colour is detected in the MCP1-chewed gum at this time, indicating that the whole dose of dye is released. In fact, the residual gum is colourless (Fig. 2c_1) and only traces of colour were detected in the 30 min-chewed gums obtained from MCP3 and MCP5 (Fig. 8); their corresponding photos did not show evident colour differences compared to MCP1-chewed gum: all the residual gums at this chewing time (30 min) are colourless (Fig. 2c_1, 2c_2 and 2c_3).

The object of this study was to obtain a formulation able to release more than 90% of the active whole dose before the chewed gum becomes colourless (that means: less than 0.06 mg of residual dye in the chewed gum for MCP formulations). The results obtained indicate that the formulations with the lower doses of dye (MCP1 and MCP3) do not achieve this aim completely because after 20 min of chewing (Fig. 7) the gums deliver almost all the colour (less

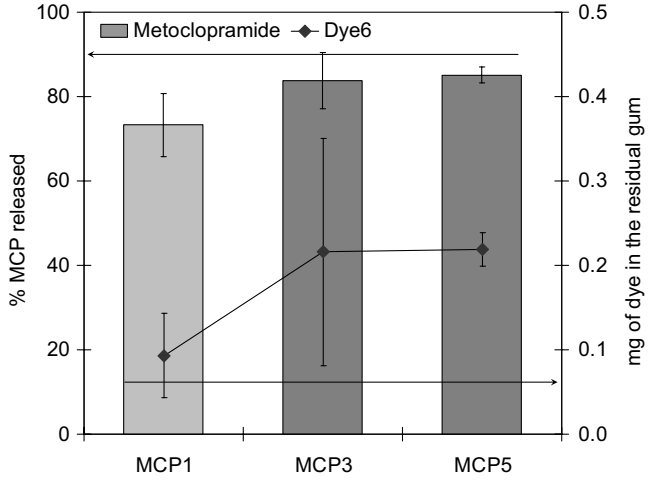

Fig. 6. Percentage of MCP released (left scale) and mg of Dye6 (right scale) in the residual-gum after 10 min of chewing (mean of volunteers).

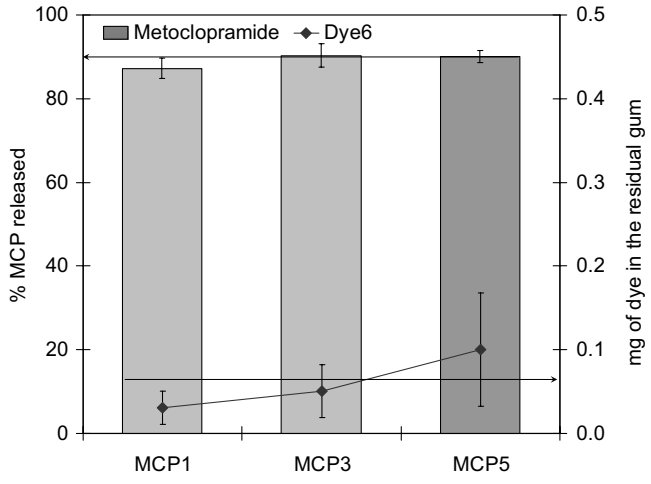

Fig. 7. Percentage of MCP released (left scale) and mg of Dye6 (right scale) in the residual-gum after 20 min of chewing (mean of volunteers).

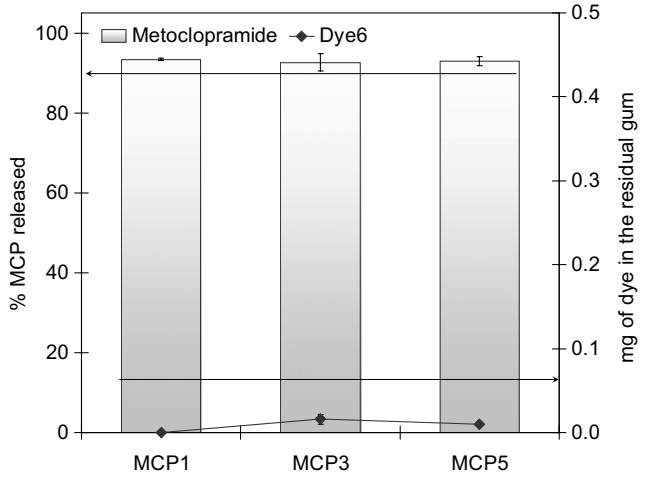

Fig. 8. Percentage of MCP released (left scale) and mg of Dye6 (right scale) in the residual-gum after 30 min of chewing (mean of volunteers).

than 0.06 mg of dye remaining in the gums) when about 90% of the active dose has been delivered. At the same chewing time, MCP5 formulation releases 90% of the active dose but the residuals are still coloured, this condition should stimulate the patient to chew the gum for a longer time. In fact this formulation produces colourless residuals (Fig. 2c_3) when more than 90% of the active has been released, that is, after 30 min of chewing (Fig. 8).

The percentages of drug released obtained from the three volunteers at 10, 20 and 30 min of chewing were plotted against the percentages of dye delivered at the same time points (Fig. 9) to evaluate the relationship between the drug and dye release rates, because many authors have demonstrated that the drug release rate from chewing gums strongly depends on the water solubility of the active [11,13,14]. The formulations containing 3 mg of Dye6: MCP3 and AAC3 were used for this purpose; the results are shown in Fig. 9. For MCP3 and ACC3 a good linear correlation between drug and dye release rates was found,

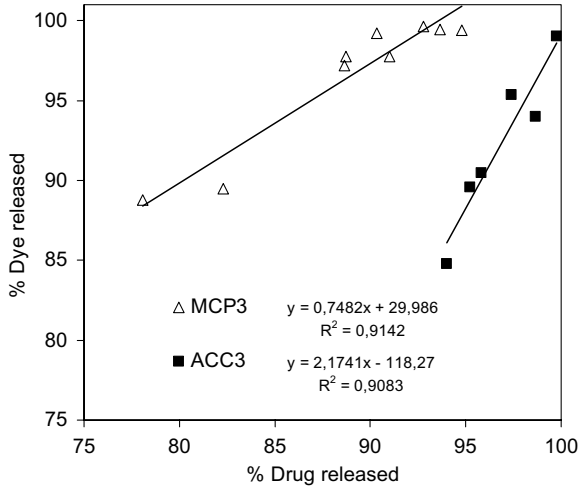

Fig. 9. Relationship between the percentages of drug and dye released form MCP3 and ACC3.

as confirmed by the correlation coefficient ($r^2 > 0.9$); these results suggest that the relationship $drug_{rate}/dye_{rate}$ for these formulations is independent of the chewing performance of volunteers.

MCP3 formulation shows a drug release rate slower than dye release rate confirmed by the slope of the regression fit (<1). The difference between the release rate of the two substances from the gum can be explained by their water solubility, in fact, MCP is less soluble in water at 25 °C (0.2 g/L) than Dye6 (190 g/L). On the contrary, the evaluation of the results obtained for ACC3 shows higher drug release rate compared to dye release rate, in fact, the slope of the regression line is >1, also in this case the water solubility of the compounds explains their different delivery during chewing, the Ascorbic Acid solubility (333 g/L) is greater than the dye solubility.

5. Conclusions

The inclusion of a proper amount of a soluble dye in a medicated chewing gum has been demonstrated to be a good approach to trace the *in vivo* drug release process from the device. In fact, the results of the chew-out study performed show how the chewing performances of the individual can influence the drug release rate from this type of formulation and confirm that a suitable dose of dye is able to indicate when the whole drug dose has been delivered from the chewing gum, independently of chewing frequency or chewing intensity. The relationship between drug and dye release rates depends on their water solubility, in fact, when the dye is more soluble than the drug, a larger dose of colour is needed to indicate the drug release process from the device. On the basis of these findings, it is possible to conclude that this therapeutic system shows a good functionality and efficiency in term of traceability of drug release during mastication from medicated chewing gum.

References

[1] Chewing gum, medicated, European Pharmacopoeia 5th edition, Suppl. 5.2 Council of Europe, Strasbourg, (2005) 3136–3137.
[2] M.R. Rassing, Specialized oral mucosal drug delivery systems, Chewing gum, in: M.J. Rathabone (Ed.), Oral Mucosal Drug Delivery, Marcel and Dekker, New York, USA, 1996, pp. 319–357.
[3] J.J. Jacobsen, L.L. Christrup, N.H. Jensen, Medicated chewing gum: Pros and Cons, Am. J. Drug. Deliv. 2 (2004) 75–88.
[4] J. Logemann, C. Smith, B. Roa Pauloski, A. Rademaker, C. Lazarus, L. Colangelo, B. Mittal, E. MacCracken, J. Gaziano, L. Stachowiak, L. Newman, Effects of xerostomia on perception and performance of swallow function, Head & Neck 23 (2001) 317–321.
[5] B. Liedberg, B. Öwall, Masticatory ability in experimentally induced xerostomia, Dysphagia 6 (1991) 212–213.
[6] R. Nemeth-Coslett, N.L. Benowitz, N.L. Robinson, J.E. Henningfield, Nicotine gum: chew rate, subjective effects and plasma nicotine, Pharmacol. Biomed. Behav. 29 (1988) 747–752.
[7] D. Hee Na, J. Faraj, Y. Capan, K.P. Leung, P.P. De Luca, Chewing gum of antimicrobial decapeptide (KSL) as a sustained antiplaque agent: Preformulation study, J. Control Rel. 107 (2005) 122–130.

[8] P. Lingström, S. Fure, B. Dinitzen, C. Fritzne, C. Klefbom, D. Birkhed, The release of vitamin C from chewing gum and its effects on supragingival calculus formation, Eur. J. Oral Sci. 113 (2005) 20–27.
[9] C. Dong, A.D. Puckett Jr., C. Dawes, The effects of chewing frequency and duration of gum chewing on salivary flow rate and sucrose concentration, Arch. Oral Biol. 40 (1995) 585–588.
[10] R. Barabolak, K. Hoerman, N. Kroll, D. Record, Chewing gum profiles in U.S. population, Commun. Dent. Oral Epidemiol. 19 (1991) 125.
[11] L. Maggi, L. Segale, S. Conti, E. Ochoa Machiste, A. Salini, U. Conte, Preparation and evaluation of release characteristics of 3TabGum, a novel chewing device, Eu. J. Pharm. Sci. 24 (2005) 487–493.
[12] European Parliament and Council Directive 94/36/EC of 30 June 1994 on colours for use in foodstuff, Official J. Eur. Commun. L 237 (1994) 13–29.
[13] L.N. Jensen, L.L. Christrup, N. Menger, H. Bundgaard, Chewing gum and lozengues as delivery systems for noscapine, Acta Pharm. Nord. 3 (1991) 219–222.
[14] M.R. Rassing, L.L. Christrup, J. Møller-Sonnergaard, Absorption of progesterone through the oral mucosa, in: Proceedings of the 47th International Congress of Pharmaceutical Sciences, Amsterdam (1987) 69.